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# Integrated Multiple-tactic Management of the Redbanded Stink Bug on Soybeans in Louisiana

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# INTEGRATED MULTIPLE-TACTIC MANAGEMENT OF THE REDBANDED STINK BUG ON SOYBEANS IN LOUISIANA

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## ABSTRACT

Stink bugs (Hemiptera: Pentatomidae) are major pests of soybeans in Louisiana and heavy infestations often lead to economic losses. The stink bug species complex has changed in the past decade with the prevalence of the invasive redbanded stink bug, *Piezodorus guildinii* (Westwood), which causes greater damage than other stink bug species. Moreover, control of the redbanded stink bug has been faced with challenges because it is less sensitive to recommended insecticides.

Therefore, studies were conducted to evaluate different control options for *P*. *guildinii*. Among the studies conducted was the current status of egg parasitoids of stink bugs in Louisiana. *Telenomus podisi* Ashmead (Hymenoptera: Platygastridae) was the predominant egg parasitoid of stink bugs. Field studies were also conducted to determine the combined effects of host plant resistance and insecticide application, on the abundance of stink bugs and their natural enemies. The soybean cultivar Pioneer 95Y20 had the least pest pressure, seed damage, and yields were higher from these plants. Insecticide application of thiamethoxam provided some control of stink bugs, although it did not impact yield. The combination of cultivar and insecticide application did not have a significant effect on natural enemies of stink bugs.

Lastly, a study was also performed to demonstrate the effect of synthetic plant volatiles on natural enemies of stink bugs in the field. Results from this study showed that methyl jasmonate had a significant impact on the number of fire ants collected during a 5-minute time interval. Overall, results from this research provide important information on the integrated pest management (IPM) of *P*. *guildinii* and other stink bugs in Louisiana.

# CHAPTER 1: INTRODUCTION AND REVIEW OF LITERATURE

## 1.1. Soybean Production and Damage

Soybean, *Glycine max* (L.) Merrill is the major oilseed crop grown worldwide and constitutes over 50% of the world's total oilseed production (USDA 2015). Soybeans are an important source of protein for human and livestock consumption (Clemente and Cahoon 2009), and biodiesel for industrial purposes (Cao et al. 2005). The United States is the major producer of soybeans (Masuda and Goldsmith 2009). Over 80 million acres of the crop was grown in the United States and about 105 million metric tons were produced in 2014 (NASS 2015). In the same year, NASS (2015) reported that soybeans were planted on approximately 1.41 million acres in Louisiana and this area produced 2.1 million metric tons.

However, production of this crop in Louisiana is negatively impacted by insect pests. Major pests of soybean include insect groups that are defoliators, stem feeders, and fruit feeders (Steffey 2015). The major defoliating species of soybeans are soybean looper *Chrysodeixis includens* (Walker) (Lepidoptera: Noctuidae), velvetbean caterpillar *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae), and green cloverworm *Hypena scabra* (F) (Lepidoptera: Erebidae) (Wier and Boethel 1995, Baur et al. 2000). The lepidopteran pests cause holes in soybean leaves and skeletonization of whole leaves can ultimately reduce seed and pod numbers (Lorenz et al. 2015). The most important stem feeder in Louisiana is the threecornered alfalfa hopper *Spissistilus festinus* (Say) (Hemiptera: Membracidae) that causes girdling on stems (Mitchell and Newsom 1984) and leads to yield loss (Stewart and Catchot 2015). Stink bug (Hemiptera: Pentatomidae) species in Louisiana are economically important fruit feeders of soybeans (Steffey 2015). The stink bug species complex in Louisiana includes the redbanded stink bug *Piezodorus guildinii* (Westwood), southern green stink bug *Nevara viridula* L., brown stink bug *Euschistus servus* (Say), green stink bug *Chinavia hilaris* (Say), *E. quadrator* Rolston, dusky stink bug *E. tristigmus* (Say), *E. ictericus* (L.), *E. conspersus* Uhler, red shouldered stink bug *Thyanta accera* McAtee, rice stink bug *Oebalus pugnax* (F) and *Edessa bifida* (Say) (Stam et al. 1987, Baur et al. 2000, Temple et al. 2013a).

Stink bugs puncture young tender seeds and this action can cause seed abortion and deformation (McPherson et al. 1994). Additionally, plant pathogens can gain entry through the puncture (Daugherty 1967, Russin et al. 1988) and cause damage that results in yield reduction or poor seed quality (McPherson et al. 1994). Reduced oil content and delayed maturity are also characteristic of stink bug damage (Boethel et al. 2000). Stink bugs also cause retention of leaves at harvest, especially when they feed on soybeans at pod development or seed filling stages (Costa and Link 1977, Panizzi et al. 1979). Overall, stink bugs are the major economic pests of soybeans because they infest a larger area of the crop in Louisiana and may exceed the economic threshold in 83 % of the infested areas (Musser et al. 2014). Furthermore, this level of infestation accounted for 45.3 % of the total insect pest losses amounting to $24.6 million in 2013 (Musser et al. 2014).

Currently, *P. guildinii* is the predominant stink bug species in Louisiana and accounts for about 54% - 65% of stink bug populations on soybeans (Temple et al. 2013a, Musser et al. 2014). This pest has also been reported to cause the highest amount of damage on soybeans in comparison to other phytophagous pentatomids (Corrêa-Ferreira and de Azevedo 2002). The extent of damage caused by *P. guildinii* could be due to the pest using some of its host plants as refuge and not food sources (Panizzi and Slansky 1985b). Therefore, the host plant range, geographical distribution, and abundance of *P. guildinii* on soybeans, life history, and control of *P. guildinii* are reviewed below.

### 1.2. Host Plants of *Piezodorus guildinii* and Seasonal Abundance on Soybeans

The cultivated host plants of *P. guildinii* include soybean *G. max*, cotton *Gossypium hirsutum* (L.), alfalfa *Medicago sativa* L., coffee *Coffea arabica* (L. ), common bean *Phaseolus vulgaris* L., and pigeon pea *Cajanus cajan* L. Millsp. (Fraga and Ochoa 1972, Panizzi et al. 2000, Baur et al 2010). On the other hand, wild host plants of *P. guildinii* include rattle pod *Crotalaria brevidens* Benth., lanceleaf rattlebox *C. lanceolata* E. Mey., hairy indigo *Indigofera hirsuta* (L.), and white clover *Trifolium repens* (L.) (Panizzi and Slansky 1985a, Panizzi and Slansky 1985b, Panizzi 1997). However, *P. guildinii* prefers feeding on soybean seeds, and colonizes soybean fields during the summer (Panizzi et al. 2000), and the wild plants are used for shelter when cultivated hosts are unavailable (Panizzi and Smith 1977, Panizzi and Slansky 1985b, Zerbino et al. 2014).

Generally, the stink bug populations in Louisiana tend to peak from June to early August on the early planted soybean (Baur 2000). Stink bugs overwinter as adults and they colonize wild hosts or crops in early planted agro-ecosystems (Yeargan et al. 1983). Soybean plants at pod fill (R5) and full pod (R6) (Fehr et al. 1971) seem to be the most suitable for *P. guildinii* and its life cycle depends on phenology of the soybean plant (Oliveira and Panizzi 2003). An increase in both nymph and adult stink bug populations were observed as the soybean progressed towards physiological maturity (Vyavhare et al. 2014, Smith et al. 2009). .

### 1.3. Geographical Distribution of *Piezodorus guildinii* in United States

The first reported occurrence of *P. guildinii* was on the Caribbean island of St. Vincent (Stoner 1922) and (Genung et al. 1964) later reported its presence in United States. Presently, *P. guildinii* is the predominant phytopgaous pentatomid in Louisiana and part of Texas (Temple et al. 2013a, Vyavhare et al. 2014). It is also present in Alabama, Arkansas, Mississippi and

Missouri (Smith et al. 2009, Tindall and Fothergill. 2011, Musser et al. 2014). The presence of *P*. *guildinii* in Louisiana was first reported by Baldwin (2004) and it was later found to exceed the economic threshold in Louisiana (Temple et al. 2013a).

Temple et al. (2013a) reported a high percentage of stink bugs in the early maturing soybean cultivars. However, *P*. *guildinii* populations were observed to have declined in the late maturing cultivars (Temple et al. 2013a). Currently, the early maturing cultivars that are classified as very early maturity, early maturity and early medium maturity are grown in Louisiana (Levy et al. 2014). The emergence of *P*. *guildinii* as the most serious stink bug pest could be due to changes in cropping system (early soybean production system) (Ashlock et al. 1998, Baur et al. 2000). Early soybean production system was initially proposed as a way to avoid high temperatures and long drought periods in the midsouth (Heatherly 1999).

### 1.4. Biology of *Piezodorus guildinii*

*P*. *guildinii* eggs are dark and spined, and usually deposited in alternating positions in two rows (Bundy and McPherson 2000), mainly on leaves of soybean (Temple 2011). Generally, the incubation period of eggs is about 8 days (Panizzi and Smith 1977). After hatching, first instar nymphs cluster around the shells and start to feed when they reach the second instar (Panizzi 1980). Second and third instars are usually gregarious with dispersal occurring throughout the field during the fourth as well as fifth instars (Panizzi 1980). The five nymphal instars have duration of 3-13 days whilst development from oviposition to adult takes an average time of about 39 days (Panizzi and Smith 1977). Additionally, the average longevity for females, and males is 41 and 34 days, respectively (Panizzi and Smith 1977). However, an interaction between photoperiod and temperature could be responsible for adult longevity. Zerbino et al. (2013) reported that adults lived for shorter days under a long photophase (14 h). In addition, adult females reared under a long photophase (14 h) showed better reproductive performance (Panizzi and Smith 1977, Zerbino et al. 2013).

### 1.5. Control of *Piezodorus guildinii*

Economic thresholds and insecticide applications are important in the management of *P. guildinii* (Davis et al. 2009, Bommiredy et al. 2006). Adequate sampling methodology is equally important in soybean IPM (Todd and Herzog 1980, Peterson 1997) and the current economic threshold for Louisiana is 4 insects per 25 sweeps (Davis 2014). Chemical control is also an important component of soybean IPM, and the recommended insecticides for the control of *P. guildinii* are acephate (Orthene, AMV Chemical Corporation, Newport Beach, CA), lambda-cyhalothrin + thiamethoxam (Endigo ZC, Syngenta Crop Protection, LLC, Greensboro, NC), bifenthrin (Brigade, FMC Corporation Agricultural Products Group, Philadelphia, PA), zeta-cypermenthrin + bifenthrin (Hero (1.24), FMC Corporation Agricultural Products Group, Philadelphia, PA), imidacloprid + cyfluthrin (Leverage, Bayer CropScience) and clothianidin (Belay, Valent Agricultural Products, Walnut Creek, CA) (Davis 2014).

Trap cropping may be effective for the management of stink bug complex species and the early planted soybeans can act as a trap crop for late planted soybeans (Baur et al. 2000). McPherson et al. 2001, Gore et al. 2006). This is because concentrating ovipositing adult stink bugs and relatively immobile nymphs in the trap crop allows chemical control to be directed only on that portion of the crop (Todd et al. 1994). McPherson (1996) reported that late maturing soybean cultivars were more likely to have higher stink bug densities and more seed damage than the earlier maturing cultivars.

Biological control agents are regarded as important natural factors in mortality of stink bugs in soybean fields (Yeargan 1979, Orr et al. 1986, Corrêa-Ferreira and Moscardi 1995, Koppel et al. 2009). In Brazil, *P*. *guildnii* was naturally controlled by different egg parasitoids by up to 42% parasitism (Corrêa-Ferreira and Moscardi 1995). The authors also reported that almost all of the recovered parasitoids belonged to Hymenoptera: Platygastridae.  *Trissolcus basalis* (Wollaston) was the predominant parasitoid followed by *Telenomus podisi* Ashmead (Corrêa-Ferreira and Moscardi 1995). Further, releasing of *T*. *basalis* negatively impacted the stink bug population, and population build-up was delayed in areas where parasitoids were released (Corrêa-Ferreira and Moscardi 1996). However, previous surveys conducted in Louisiana on parasitoids of stink bugs were conducted before *P*. *guildinii* emerged as an important pest. Orr et al. (1986) found that *T*. *podisi* was the predominant parasitoid species and the varying parasitism rates were lower than 50% on *N*. *viridula, Euschistus* spp., *C*. *hilaris*, and *E*. *bifida*. However, natural enemies like parasitoids can be negatively affected by non-selective insecticides (Panizzi 2013). On the other hand, natural enemies like parasitoids and predators can be conserved by application of synthetic plant volatiles (Moraes et al. 2008, Vieira et al. 2013).

Other methods of control that have been evaluated include soybean plant resistance through antixenosis and antibiosis (McPherson et al. 2007, Silva et al. 2013, Silva et al. 2014). From the 17 soybean entries evaluated for antixenosis, eight cultivars showed low preference for stink bug pod feeding (Silva et al. 2014). These eight soybean cultivars that demonstrated antixenosis also had the lowest numbers of probes by stink bugs (Silva et al. 2014). In antibiosis evaluation study, 10 soybean cultivars had low percentage survival of nymphs and some of these cultivars extended nymph developmental time (Silva et al. 2013).

### 1.6. Insecticide Interaction with *Piezodorus guildinii*

Some populations of *P*. *guildinii* in Louisiana have been reported to be less susceptible to organophosphates, and pyrethroids because the lethal concentration 50 ($LC_{50}$) of these chemicals increased between early and late summer (Baur et al. 2010). Equally, an increase in esterase activity was observed during the same period. Esterase activity has been associated with resistance against organophosphates (Harold and Ottea 2000). However, *P*. *guildinii* treated with high rates of pyrethroids were susceptible (Baur et al. 2010). Castiglioni et al. (2008) reported that *P*. *guildinii* from commercial crops (with repeated applications of the same insecticide) usually become less susceptible to these chemical products.

In another study, *P*. *guildinii* was also less susceptible with varying degrees to different organophosphates and pyrethroids in comparison to *N*. *viridula* (Temple et al. 2013b). It was also reported that *P*. *guildinii* was 2-8 fold and 4-8 fold less sensitive to organophosphates and pyrethroids, respectively. Temple et al. (2013b) also reported that combinations of pyrethroids and organophosphates provided the greatest amount of control. The combination of pyrethroids and neonicotinoids also provided considerable amount of control (Temple et al. 2013b). The development of tolerance to insecticides is of great concern to both growers and researchers.

### 1.7. Justification of Study

The heavy reliance on insecticides has led to *P*. *guildinii* becoming less susceptible to chemical control as mentioned above. The overall goals of the present studies were to evaluate different control tactics of *P*. *guildinii* with the aim of reducing heavy reliance on insecticides in Louisiana. Evaluating effects of natural enemies and their enhanced abundance by host plant resistance, insecticides, and plant volatiles on *P*. *guildinii* would be relevant in providing soybean growers with alternative management tactics. Therefore, three objectives were proposed to achieve these goals.

## 1.8. Study Objectives

i. To determine the current status of stink bug egg parasitoids and percentage of parasitism in Louisiana.

ii. To evaluate effects of host plant resistance, insecticides, and biological control on *P. guildinii* and other stink bug species.

iii. To determine effects of exogenous application of methyl jasmonate on natural enemies of stink bugs.

# CHAPTER 2: CURRENT STATUS OF PARASITOIDS OF STINK BUG (HEMIPTERA: PENTATOMIDAE) EGGS ON SOYBEANS IN LOUISIANA

## 2.1. Introduction

Soybean, *Glycine max* (L.) Merrill is an important crop in Louisiana with an estimated harvest area of a million acres (Baldwin et al. 1997, NASS 2015). Growers can increase profits from soybean production by decreasing production costs and increasing yields (Boquet 1998, Heatherly and Spurlock 1999). However, production costs can increase due to repeated attempts to reduce pest pressure on soybeans in the field (Guidry 2010). Stink bugs (Hemiptera: Pentatomidae) are among the important insect pests of soybeans in the southern United States because they cause the most economic losses (Tynes and Boethel 1996, Musser et al. 2014). Stink bugs use their piercing and sucking mouthparts to obtain nutrients from soybean seeds (McPherson and McPherson 2000). This mode of feeding can cause seed deformation and the feeding punctures can be used as entry points for pathogens, thus resulting into reduced soybean seed quality and yields (Yeargan 1977, McPherson at al. 1994).

The economically important stink bugs in Louisiana include the redbanded stink bug, *Piezodorus guildinii* (Westwood), southern green stink bug, *Nezara viridula* L., green stink bug, *Chinavia hilaris* (Say) and the brown stink bug species complex (*Euschistus* spp.) including *Euschistus servus* (Say) (Jensen and Newson 1972, Tynes and Boethel 1996, Temple et al. 2013a). However, *P. guildinii*, a native of Central America (Panizzi and Slansky 1985), is currently the predominant stink bug in Louisiana (Temple et al. 2013a) and thus control tactics should be aimed at this pest. This species has been reported to cause more damage than the other stink bug pests associated with soybeans (Corrêa Ferreira and de Azevedo 2002).

Sampling, action thresholds and insecticide applications are the major control tactics used for the control of phytophagous pentatomids (Boethel 2004, Musser et al. 2014). However, *P. guildinii* was reported to be less sensitive to certain classes of insecticides in comparison to other stink bug species (Temple et al. 2013b). Therefore, other alternative management tactics of *P. guildinii* need to be investigated. Biological control is an option that can be used to cause stink bug mortality (Corrêa Ferreira and Moscardi 1996). Egg parasitoids are important biological control agents that cause natural mortality of stink bug eggs in soybeans (Yeargan 1979, Orr et al. 1986, Koppel et al. 2009). Most egg parasitoids of stink bugs belong to the genera *Trissolcus* Ashmead and *Telenomus* Haliday (Hymenoptera: Platygastridae) (Johnson 1984b).

In Brazil, *Telenomus podisi* Ashmead was reported to parasitize over 50% of *P. guildinii* and *N. viridula* eggs (Corrêa-Ferreira and Moscardi 1995). In addition, a study conducted to examine integrated pest management through the releasing *Trissolcus basalis* (Wollaston) on soybean resulted in stink bug population reductions of over 50%. In the United States, it was also reported that *T. podisi* was the most prevalent egg parasitoid (Yeargan 1979, Orr et al. 1986, Koppel et al. 2009). However, this study was conducted before *P. guildinii* became an important pest of soybeans in Louisiana (Temple et al. 2013a). Currently, the impact of egg parasitoids on *P. guildinii* in Louisiana is unknown, and this study provides useful information on the indigenous natural enemies and their impact on the pest. Therefore, the objectives of the present study were to; (1) determine parasitism of stink bug eggs in different locations within Louisiana; and (2) determine parasitism within the soybean vertical strata and plant structures.

## 2.2. Materials and Methods

2.2.1. Stink bug egg parasitism across geographical locations in Louisiana**.**

This study was conducted at two Louisiana State University Agricultural Center Research Stations (LSU AgCenter) from 2008 to 2010. The study sites were located in Alexandria (Dean Lee Research Station) and Bossier City (Red River Research Station) (Figure 2.1). Soybean cultivars, Asgrow 4404 (Monsanto, St. Louis, MO) and Asgrow 5905 (Monsanto, St. Louis, MO) were planted in adjacent blocks of~5 acres and managed according to the recommended agronomic practices with no insecticide application. Sampling of soybean plants for stink bug egg clusters from each cultivar was performed weekly from pod fill (R5) to maturity (R7) (Fehr et al. 1971). Stink bug egg clusters were collected in 9.1 m of row which was alternated weekly. Clusters were identified to species as described by Bundy and McPherson (2000). Stink bug egg clusters were then placed separately in a 5 cm clear plastic container (Wide-mouth jars; Uline, Pleasant Prairie, WI) that contained grade one 9 cm sheet of cellulose filter paper dampened with distilled water at the bottom (Whatman Inc., Sanford, ME).

Each container with an egg cluster was labeled according to geographical location, collection date, stink bug species, and number of eggs per cluster, and later kept in an environmental chamber (model I-36VL, Percival Scientific, Perry, IA) at 25 ± 1°C, 60 ± 10% RH and a photoperiod of 14:10 (L:D). Egg clusters of *N. viridula* and *C. hilaris* were grouped together and hereafter called “green complex” and all *Euschistus* spp. were categorized as “brown complex” (Temple 2011). Stink bug nymph or adult parasitoid emergence was monitored and recorded daily. The number of emerged parasitoids was obtained to compute the percentage of egg parasitism [(number of parasitized eggs / number of eggs per cluster) *100]. Parasitoids from each stink bug egg cluster were preserved separately in vials containing 95% ethyl alcohol.

Thereafter, individual parasitoids were air dried, mounted on triangular card points, and identified to species using taxonomic keys (Masner 1980, Johnson 1984a, 1984b, 1985a, 1985b, 1987).

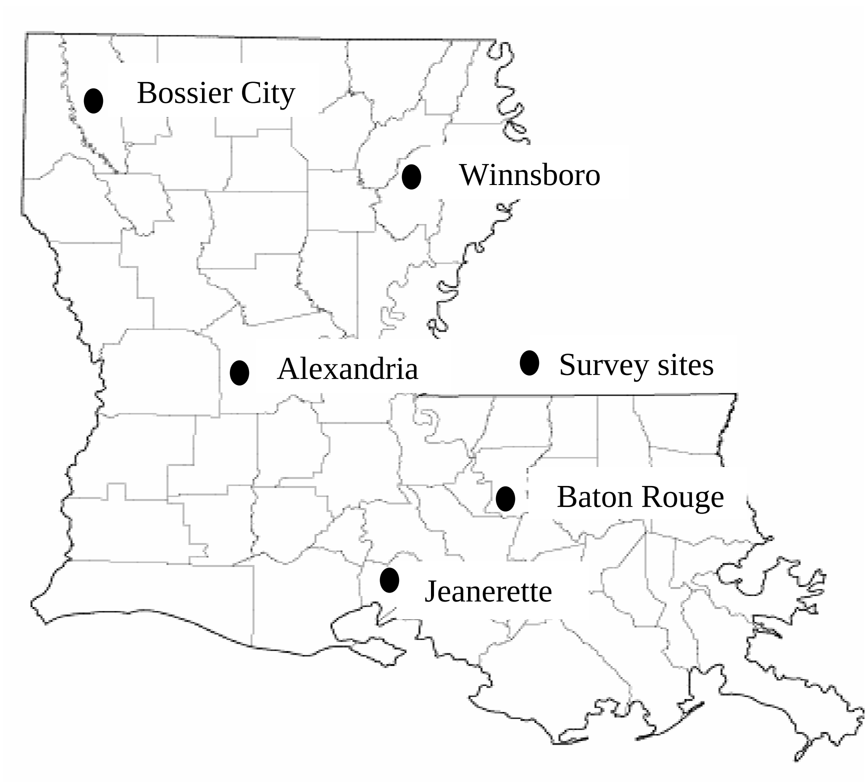



Figure 2.1. Survey sites at different locations in Louisiana

2.2.2. Stink egg parasitism within the soybean vertical strata and plant structures.

Study sites were located at three LSU AgCenter Research Stations in Baton Rouge (Ben Hur Research Farm), Jeanerette (Iberia Research Station), and Winnsboro (Macon Ridge Research Station) (Figure 2.1). Four soybean cultivars, Pioneer 93Y90 (DuPont, Johnston, IA), Asgrow 4606 (Monsanto, St. Louis, MO), Asgrow 5606 (Monsanto, St. Louis, MO), and Asgrow 6702 (Monsanto, St. Louis, MO), were each planted in adjacent blocks measuring~0.1 ha. Each block was sub-divided into equal six sub-plots.

Weekly sampling of soybean plants in each sub-plot for stink bug egg clusters began at pod development stage (R3) and ended at maturity (R7) (Fehr et al. 1971). Five plants were randomly collected by destructive sampling from each sub-plot on two adjacent rows (the sampled rows were alternated every week in each plot). Height (cm) and number of nodes on each soybean plant were obtained to categorize soybean vertical strata (lower, middle, and upper canopy). Number of stink bug eggs per cluster, location in the soybean canopy, location on plant structures (abaxial, and adaxial surface of leaves, pod, or stem) were recorded, and identified to species according to Bundy and McPherson (2000). Egg clusters of *N. viridula* and *C. hilaris* were grouped together and hereafter called "green complex" and all *Euschistus* spp. were categorized as "brown complex" (Temple 2011).

Each stink bug egg cluster was placed separately in a 5 cm clear plastic container (Wide-mouth jars; Uline, Pleasant Prairie, WI) that contained wet grade one 9 cm sheet of cellulose filter paper at the bottom (Whatman Inc., Sanford, ME). All egg clusters were reared in an environmental chamber (model I-36VL, Percival Scientific, Perry, IA) at 25 ± 1°C, 60 ± 10% RH and a photoperiod of 14:10 (L:D) at LSU AgCenter, Baton Rouge, LA. Emergence of stink bug nymphs or parasitoids was monitored daily. Percentage of egg parasitism was computed as [(number of parasitized eggs / number of eggs per cluster) *100]. Parasitoids from each stink bug egg cluster were preserved separately in vials containing 95% ethyl alcohol. Thereafter, individual parasitoids were dried, attached to triangular card points and identified to species using diagnostic keys (Masner 1980, Johnson 1984a, 1984b, 1985a, 1985b, 1987). Adult female parasitoids were distinguished from males using diagnostic features described by Johnson (1984b) to determine the sex ratio (male / male + female). Some specimens were sent to The

Ohio State University Museum of Biological Diversity (Columbus, OH) for identification confirmation.

2.2.3. Data Analysis.

Data on the number of stink bug eggs per cluster and percentage of egg parasitism from 2008-2010 was checked for normality with Shapiro-Wilk's test (PROC UNIVARIATE) and homogeneity of variance with Levene's test (PROC GLM) (SAS Institute 2013). Numbers of stink bug eggs per cluster were log transformed and percentage of egg parasitism was arcsine transformed when statistical assumptions of normality were not met. Data for locations and stink bug species were then subjected to one-way analysis of variance (ANOVA) in PROC MIXED, and means were separated by Tukey's honestly significant difference (HSD) test with $P = 0.05$ (SAS Institute 2013). Analysis was only done on brown complex, green complex, and *P. guildinii* due to low sample size.

For the 2009-2011 data, Shapiro-Wilk's tests in PROC UNIVARIATE and Levene's test in PROC GLM were performed for normality of data and homogeneity of variance, respectively (SAS Institute 2013). Data was normalized by log transformation for number of eggs per cluster. Percentage of egg parasitism and percentage of parasitoid species among stink bug species were arcsine transformed when necessitated. One-way ANOVA was performed to analyze data in PROC MIXED and means were separated by Tukey's HSD test at $\alpha = 0.05$ (SAS Institute 2013). Sex ratio within parasitoid species was analyzed by *t*-test (Mitchell et al. 2004) using PROC TTEST and their means with standard error (SE) were obtained in PROC MEANS (SAS Institute 2013). Analysis on percentage of parasitoid species among stink bugs and sex ratio were only performed on three out of the four parasitoid species that emerged due to small sample size.

Further analysis of the 2009-2011 data was conducted by categorizing oviposition site (leaf [abaxial or adaxial] or pod) and egg cluster location in the vertical plant strata, and calculating differences in egg cluster parasitism between categories using multinomial logistic regression and the statistical program R (R Core Team 2015). The likelihood ratio test (LRT) was used to test for significance. This analysis also allowed the calculation of the odds ratio (OR), a way of comparing whether the probability of a certain event is the same for two groups. The OR is the base of the natural logarithm, *e*, raised to the power of the logit. Due to low sample size, analysis could only be done on *P. guildinii*.

**2.3. Results**

2.3.1. Stink bug egg parasitism across geographical locations in Louisiana.

Number of stink bug eggs per cluster did not vary significantly by location ($F = 0.41$; df = 3; $P = 0.7426$). However, significant differences in eggs per cluster were observed among stink bug species ($F = 69.68$; df = 4; $P < 0.0001$). Green complex had the highest number of eggs per cluster (Table 2.1). Percentage of egg parasitism was significantly higher in Alexandria (34.1 ± 4.1%) than Bossier City (14.9 ± 3.7%) ($F = 9.4$; df = 1; $P = 0.0024$). Percentage of egg parasitism was significantly higher on brown complex and least on green complex in Alexandria ($F = 7.16$; df = 2; $P = 0.0012$) (Table 2.1). The percentage of parasitized brown complex eggs was also significantly higher in Bossier City ($F = 4.78$; df = 2; $P = 0.0019$).

Of the egg parasitoids that emerged, *T. podisi* accounted for 84.8%, followed by 13.9% of *T. euschisti*, and 1.3% of *T. sanctivincenti* Ashmead at Alexandria in 2008. *T. podisi* constituted 86.6% of egg parasitoids collected from *P. guildinii* and 13.4% were collected from brown complex. *Tr. euschisti* and *T. sanctivincenti* Ashmead were exclusively collected from *P. guildinii* eggs. In 2009, *T. podisi* accounted for 90.7% of the total egg parasitoids and 9.3% were

*T*. *consimilis* at Alexandria. At this location, *T*. *podisi* made up 54.1% of parasitized brown complex eggs, followed by 17.8% from *P*. *guildinii*, 16.2% from the predatory spined soldier bug *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae), and 2.7% from green complex. *T*. *consimilis* Ashmead was exclusively collected from brown complex eggs. In Bossier City, *T*. *podisi* made up 89.1% of the total egg parasitoids and the rest was *Tr*. *basalis* at 10.9% in 2009. *T*. *podisi* emerged from 54.1% of parasitized brown complex eggs and 45.9% of parasitized *P*. *guildinii* eggs. All *Tr*. *basalis* were collected from *P*. *maculiventris*.

Table 2.1. Mean eggs per cluster and percentage of eggs parasitized per egg cluster (%) among stink bug species from 2008 – 2010

| | Eggs per cluster ±SE | % of eggs parasitized ± SE | |
|---|---|---|---|
| | | Alexandria | Bossier City |
| Brown complex | 19.9 ± 0.9b | 46.5 ± 6.7a | 24.3 ± 7.6a |
| Green complex | 64.6 ± 7.0a | 7.5 ± 6.8b | 0.0 ± 0.0b |
| *P*. *guildinii* | 18.0 ± 0.8b | 28.3 ± 5.4b | 11.7 ± 4.7ab |

Means followed by same letter within columns are not significantly different ($P > 0.05$; Tukey's HSD)

2.3.2. Stink egg parasitism within the soybean vertical strata and plant structures.

A total of 4,621 stink bug eggs in 240 egg clusters were sampled and characterized over three years. Of these, 185 egg clusters (77.1%) were from *P*. *guildinii*, 23 (9.6%) from brown complex, 19 (7.9%) from *P*. *maculiventris*, and 13 (5.4%) from green complex. The mean number of eggs per cluster differed by species ($P < 0.0001$) with the green complex having the most eggs per cluster (Table 2.2). The percentage of eggs parasitized per egg cluster also differed between species ($P = 0.0152$) with *P*. *maculiventris* having the most parasitized eggs per cluster (Table 2.2).

Table 2.2. Mean eggs per cluster and percentage of eggs parasitized per egg cluster (%) between stink bug species from 2009 – 2011

| | Eggs per cluster ± SE | % of eggs parasitized ± SE |
|---|---|---|
| Brown complex | 16.4 ± 1.4b | 29.9 ± 8.3b |
| Green complex | 52.7 ± 5.8a | 13.8 ± 8.7b |
| *P. guildinii* | 17.6 ± 0.5b | 33.8 ± 3.1ab |
| *P. maculiventris* | 16.1 ± 1.8b | 63.4 ± 9.5a |

Means followed by same letter within columns are not significantly different ($P > 0.05$; Tukey's HSD)

Vertical plant strata by oviposition site interactions were not significant ($P = 0.3959$). Focus was then placed on single factors. Parasitized egg clusters were evenly distributed throughout the soybean canopy ($P = 0.5906$). However, oviposition site was significant ($P = 0.0005$) with more parasitism occurring on leaves (39.0 ± 4.4%) than pods (27.7 ± 4.4%). The OR for leaves was 2.59. Thus, the odds for egg clusters on leaves to be parasitized are 2.59 times greater than the odds for egg clusters on pods. Further analysis on leaf side (abaxial vs. adaxial) showed that there was no parasitism preference for either side ($P = 0.8910$); abaxial parasitism was 39.2 ± 6.0% and adaxial was 38.0 ± 6.5%.

During this study, *T. podisi* accounted for 95.8 ± 3.8% of the total parasitoids and it was significantly higher than the other parasitoids which included *Tr. euschisti* (3.0 ± 1.5%), *Gryon obesum* Masner (Hymenoptera: Plastygastridae) (1.2 ± 0.9%), and *T. longicornis* Johnson (0.7 ± 0.4%) ($F = 358.7$; df = 3; $P < 0.0001$). Sex ratio was highly female biased for *T. podisi* and *Tr. euschisti* except for *G. obesum* (Table 2.3).

*P. guildinii* had a significantly higher percentage of eggs parasitized by *T. podisi* ($F = 63.8$; df = 3; $P = 0.0005$) (Table 2.4). *Tr. euschisti* emergence was significantly higher on *P.*

*maculiventris* ($F$ = 162.3; df = 3; $P$ < 0.001) (Table 2.4). Parasitized *P. guildinii* eggs had significantly higher emergence of *G. obesum* ($F$ = 69.1 df = 3; $P$ < 0.0001).

Table 2.3. Sex ratio of egg parasitoid species (± SE)

| | *T. podisi* | *Tr. euschisti* | *G. obesum* |
|---|---|---|---|
| Female | 0.8 ± 0.0 | 0.9± 0.0 | 0.4 ± 0.4 |
| Male | 0.2± 0.0 | 0.1 ± 0.0 | 0.6± 0.4 |
| *t* | 285.0 | 33.4 | 25.0 |
| *P* | < 0.0001 | 0.0030 | < 0.0001 |

Table 2.4. Mean composition of parasitoid species among stink bug species from 2009 - 2011

| | *T. podisi* | *Tr. euschisti* | *G. obesum* |
|---|---|---|---|
| Brown complex | 5.3± 2.4c | 0.0 ± 0.0c | 0.0 ± 0.0c |
| Green complex | 3.5 ± 2.4c | 0.0 ± 0.0c | 0.0 ± 0.0c |
| *P. guildinii* | 65.0 ± 1.3a | 30.8 ± 4.4b | 93.3 ± 16.6a |
| *P. maculiventris* | 22.8 ± 6.1b | 69.2 ± 5.4a | 6.7 ± 0.6b |

Means followed by same letter within columns are not significantly different ($P$ > 0.05; Tukey’s HSD)

### 2.4. Discussion

The percentage of parasitized eggs ranged from 11.7 to 63.4% in both studies. These results are comparable to parasitism that was reported earlier in Louisiana and other States (Yeargan 1979, Orr et at, 1986, Koppel et al. 2009, Tillman 2011). During the 2008–2010 study, percentage of parasitized brown complex eggs was higher than green complex and yet the former species had fewer eggs per cluster. Percentage of egg parasitism appears to increase with the decrease in number of eggs per cluster (Colazza and Bin 1995, Tillman 2010). Yeargan (1979) suggested that lower egg parasitism on green complex was mainly due to the parasitoids' inability to parasitize all eggs in each cluster. In addition, it was proposed that egg parasitoids were likely to parasitize egg clusters with fewer eggs due to reproductive behavior (Waage 1982, Higuchi 1993). However, the present study also showed that percentage of egg parasitism on *P. guildinii* was lower than brown complex despite having similar number of eggs per cluster at both locations. Historically, brown complex eggs experienced the highest percentage of parasitism in Louisiana (Orr et al. 1986). It is possible that the greater percentage of parasitism on brown complex eggs in this study could be maintaining this stink bug species at low levels. This proposal is collaborated by low levels of brown complex sampled from different locations in Louisiana (Temple et al. 2013a).

The differences in percentage of egg parasitism between locations may have due to stink bug species composition which in turn can impact parasitism. Temple et al. (2013a) reported that adult *P. guildinii* was the predominant stink bug species in Alexandria from 2008 to 2010. They also showed that the stink bug species dominance switched between green complex and brown complex in Bossier City during this period. It is likely that the reported dominance of green complex in Bossier City could have decreased egg parasitism in the present study. Stink bugs with high number of eggs per cluster have been reported to decrease parasitism (Yeargan 1979, Tillman 2010). In addition, low levels of *P. guildinii* were reported in Bossier City compared to southern locations in Louisiana (Temple et al. 2013a). The current study also showed that *P. guildinii* had higher percentage of egg parasitism in comparison to green complex. It is possible that higher percentage of egg parasitism would have been observed if either brown complex or *P. guildinii* was consistently predominant in Bossier City.

The reason for higher percentage of parasitism on *P. maculiventris* during the second part of the study is not clear. It was reported that parasitoids have a tendency to parasitize frequently encountered host species (Orr et al. 1986). However, naturally occurring *P*. *maculiventris* egg clusters are usually fewer in comparison to other stink bug species (Koppel et al. 2009). A plausible explanation is that other mechanisms were involved in the high percentage parasitism of *P*. *maculiventris* eggs. Nevertheless, percentage parasitism of *P*. *guildinii* eggs was statistically similar to *P*. *maculiventris*. This would be expected because *P*. *guildinii* is the predominant stink bug species in Louisiana (Temple et al. 2013a). In addition, percentage parasitism of *P*. *guildinii* eggs exceeding 50% was reported in its native region (Corrêa-Ferriera and Moscardi 1995, Corrêa-Ferriera and Moscardi 1996).

This study also demonstrated that parasitoids preferentially parasitized stink bug eggs on leaves. Previous reports have shown that egg parasitoids of hemipteran pests can attack a higher number of host eggs on leaves compared to other plant structures. For example, egg parasitism of the bean bugs, *Riptortus sclavatus* (Fabricius) (Hemiptera: Alydidae) and *R. pedestris* by the parasitoid *Ooencyrtus nezarae* Ishii (Hymenoptera: Encyrtidae) was greater on leaves than pods (Takasu et al. 1998, Kim and Lim 2010). Takasu et al. (1998) suggested that parasitoids might preferentially select certain microhabitats. This would imply that parasitoids in the current study preferred to search for host stink bug eggs on leaves. In addition, physical traits of different structures on a plant can lessen host searching abilities by parasitoids (Godfray 1994, Takasu et al. 1998). The preference for host eggs on leaves can have implications in the abundance of stink bugs in Louisiana. Brown complex, green complex, and *P*. *maculiventris* deposit their eggs mainly on soybean leaves (Bundy and McPherson 200, McPherson and McPherson 2000). This behavior can positively influence egg parasitism. However, *P*. *guildinii* can oviposit on soybean pods during pod fill (R5) to maturity (R7) stages in some soybean cultivars (Temple et al. 2016). This behavior can reduce parasitism of eggs and would explain the prevalence of *P*. *guildinii* in Louisiana.

This is the first report on naturally occurring egg parasitoids of *P*. *guildinii* on soybeans in Louisiana. *T*. *podisi* consistently parasitized all stink bug species collected in the present study. This parasitoid has been reported to be a generalist (Yeargan 1979, Corrêa-Ferriera and Moscardi 1995, Koppel et al. 2009, Tillman 2010, Abram et al. 2013, De Almeida et al. 2015). However, *T*. *podisi* favorably parasitized *P*. *guildinii* in the current study. *Tr*. *basalis* and *Tr*. *euschisti* are also generalist parasitoids (Orr et al. 1986, Okuda and Yeargan 1988, Corrêa-Ferriera and Moscardi 1995), although *P*. *maculiventris* appeared to be a favored host. On the contrary, *G*. *obesum* is usually collected from *Euschistus* spp. and *N*. *viridula* eggs (Buschmann and Whitcomb 1980, Corrêa-Ferriera and Moscardi 1996, Ehler 2002) but it parasitized *P*. *maculiventris* and *P*. *guildinii* in the present study. The emergence of *T*. *sanctivincenti* and *T*. *longicornis* from *P*. *guildinii* was an unexpected observation because these hosts were previously reported to have unknown hosts (Johnson 1984b). In addition, *G*. *obesum*, *T*. *longicornis*, and *T*. *consimilis* collected in the study were not previously documented on stink bug eggs in Louisiana. Therefore, it can be postulated that the introduction of *P*. *guildinii* to the stink bug species complex in Louisiana has led to parasitization by uncommon egg parasitoids.

*T*. *podisi*, *Tr*. *euschisti*, and *T*. *longicornis* had a sex ratio that was female biased. The proportion of females to males is usually high in most *Telenomus* spp. and *Trissolcus* spp. (Hirose et al. 1996, Olaye et al. 1997, Chabi-Olaye et al. 2001, Foerster and Doetzer 2006, Paz-Neto 2015). This has been attributed to mechanisms related to reproductive behavior of some Platygastridae species (Waage 1982). This factor probably accounts for the prevalence of *T*.

*podisi* in the present study. The male biased sex ratio of *G. obesum* in the present study was different from reports of high female proportions observed in other *Gryon* spp. (Vogt and Nechols 1993, Mitchell et al. 2004, Peverieri et al. 2012). However, the reason for high male proportions of *G. obesum* in the current study is unknown.

This current study demonstrated the differences in parasitism between stink bugs. It also showed the variations in stink bug egg parasitism between oviposition sites on soybeans. Evidently, the species composition of stink bug egg parasitoids has changed from the last report by Orr et al. (1986). The results from this study are timely because of the current prevalence of *P. guildinii* in Louisiana (Temple et al. 2013a). This pest was reported to be more destructive on soybean in comparison with other phytophagous pentatomids (Corrêa Ferreira and de Azevedo 2002). Therefore, soybean growers have to contend with increased production costs from chemicals to reduce damage by *P. guildinii*. On the other hand, results from this study can contribute in increasing stink bug mortality by natural enemies. A successful biological control program was documented in Brazil when stink bug populations were significantly reduced after inoculatively releasing egg parasitoids (Corrêa-Ferreira and Moscardi 1996). Scouting and insecticide application are still the primary management tactics in Louisiana but information on the current status of egg parasitoids can be integrated in potentially compatible insecticide and biological control programs.

# CHAPTER 3: COMBINING HOST PLANT RESISTANCE AND INSECTICIDE APPLICATIONS TO CONTROL STINK BUGS IN SOYBEAN

## 3.1. Introduction

Soybean, *Glycine max* (L.) Merrill is grown on slightly over one million acres in Louisiana and thus, it is important to the state's economy (Baldwin et al. 1997, Musser et al. 2014). However, considerable production losses are incurred due to insect pest infestations (Musser et al. 2014). Insects of economic importance in Louisiana include the stink bug species complex (Hemiptera: Pentatomidae), soybean looper *Chrysodeixis includens* (Walker) (Lepidoptera: Noctuidae), velvetbean caterpillar *Anticarsia gemmatalis* (Hübner) (Lepidoptera: Noctuidae), green cloverworm *Hypena scabra* (F.) (Lepidoptera: Erebidae), and the threecornered alfalfa hopper *Spissistilus festinus* (Say) (Hemiptera: Membracidae) (Mitchell and Newsom 1984, Russin et al. 1987, Baur et al. 2000, Boethel et al. 2000, Temple et al. 2013a).

However, stink bugs cause the most economic losses in soybean by reducing yield and increasing production costs (Musser et al. 2014). The economically important stink bug species in Louisiana include the green stink bug *Chinavia hilaris* (Say), southern green stink bug *Nezara viridula* (L.), brown stink bug *Euchistus servus* (Say), and the redbanded stink bug *Piezodorus guildinii* (Westwood) (Tynes and Boethel 1996, McPherson and McPherson 2000, Temple et al. 2013a). The redbanded stink bug causes more damage than other phytophagous pentatomids (Corrêa-Ferreira and de Azevedo 2002) and is currently the main stink bug species of soybeans in Louisiana (Temple et al. 2013a).

Stink bugs feed use their piercing and sucking mouthparts to obtain nutrients from soybean seeds (McPherson and McPherson 2000). This kind of feeding leads to empty pods (Corrêa-Ferreira and de Azevedo 2002), deformed seeds (McPherson et al. 1994) and retention of green leaves at harvest (Vyavhare et al. 2015). Punctures made by stink bugs can also predispose soybean seeds to invasion by fungi (Kilpatrick and Hartwig 1955, Daugherty 1967). Damage by different stink bug species can be reduced by applying several control strategies aimed at suppressing stink bug populations and these can be grouped under the broad categories of economic thresholds (Kogan and Turnipseed 1987), insecticide applications (Willrich et al. 2003, Temple et al. 2013b), cultural control (Todd et al. 1994, McPherson and Newsom 1984), host plant resistance (Boethel 1999, Campos et al. 2010, Michereff et al. 2015), and biological control (Stam et al. 1987, Orr et al. 1986, Koppel et al. 2009).

The commonly used control methods for redbanded stink bug in Louisiana are economic thresholds (LSU AgCenter 2015) and chemical applications (Baur et al. 2010, Temple et al. 2013b). Unfortunately, redbanded stink bug populations in the United States have been reported to show less sensitivity to insecticides in comparison with populations in Brazil (Baur et al. 2010). However, developing an integrated pest management (IPM) program for soybeans can immensely assist soybean growers by reducing insecticide use of up to 50% (Corrêa-Ferreira et al. 2000, Panizzi 2013). Several studies have been conducted to demonstrate the compatibility between different control methods of stink bugs (Orr et al. 1986, Abudulai et al. 2001, Kilpatrick et al. 2005, Michereff et al. 2015, Turchen et al. 2015). In some studies, mortality of predators and egg parasitoids of stink bugs was relatively low after exposure to selective insecticides (Kilpatrick et al 2005, Turchen et al. 2015).

Currently, little is known about the integration of management tactics such as host plant resistance with insecticide application and biological agents to control redbanded stink bug in Louisiana. Some commercial soybean cultivars in the United States are known to be moderately resistant to southern green stink bugs (Boethel 1999). However, these cultivars are not grown on a large scale due to their low yield (Lambert and Tyler 1999). Biological control agents of stink bugs include the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae) and egg parasitoids (Hymenoptera: Platygastridae) (Stam et al. 1987, Orr et al. 1986). Egg parasitism by platygastrid parasitoids was studied before redbanded stink bug become the predominant phytophagous pentatomid in Louisiana.

The present study was conducted to evaluate partial host plant resistance in combination with insecticide and biological control for the control of redbanded stink bug and other stink bugs species. Therefore, objectives of this study were; (1) to determine the impact of soybean cultivars and insecticides on stink bug abundance; (2) to determine the impact of soybean cultivars and insecticides on soybean seed weight, seed damage, and yield; and (3) to determine the effect of soybean cultivars and insecticides on abundance of red imported fire ants and egg parasitoids.

## 3.2. Materials and Methods

### 3.1.1. Experimental locations.

This study was done at Louisiana State University Agricultural Center Research Stations (LSU AgCenter) in 2011, 2012 and 2013 (Figure 3.1). In 2011, the study was carried out in Baton Rouge (Ben Hur Research Farm). The experiments in 2012 and 2013 were conducted in Alexandria (Dean Lee Research Station) and Baton Rouge (Ben Hur Research Farm).

### 3.1.2. Soybean cultivars and field design.

In 2011, soybean cultivars Deltapine (DP) 4888 RR (Delta &Pine Land Technology Holding Company, LLC Scott, MS) and PR 4906 RR (Progeny Ag Products, Wynne, AR) were each planted in plots measuring 7.6 m long x 4 rows wide. In 2012, Pioneer 95Y20 (DuPont, Johnston, IA) and PR 4906 RR (Progeny Ag Products, Wynne, AR) were also planted in plots measuring 7.6 m long x 4 rows wide. In 2013, Asgrow (AG) 5332 (Monsanto, St. Louis, MO), DP 4888 RR (Delta & Pine Land Technology Holding Company, LLC Scott, MS), Pioneer 93Y92 (DuPont, Johnston, IA), Pioneer 95Y20 (DuPont, Johnston, IA), Pioneer 95Y40 (DuPont, Johnston, IA), and Progeny PR 4906 RR (Progeny Ag Products, Wynne, AR) were planted in plots measuring 7.6 m long x 22 rows wide. All the cultivars were managed according to the recommended agronomic practices (Levy et al. 2016). Four insecticides and an untreated check (UTC) were randomly assigned to soybeans plots when redbanded stink bug populations reached the economic threshold (4 insects/25 sweeps) with the exception of plots in 2011. In 2012, each plot was randomly assigned a treatment and insecticides were applied on all four rows per plot. In 2013, each treatment was applied to a 2-row wide sub-plot within each plot. All five treatments (insecticides or UTC) were randomized in a block design and replicated four times.

The four insecticides selected were acephate (Orthene 97, AMVAC, Los Angeles, CA) at 0.84 kg AI/ha, flonicamid (Carbine 50 WG, FMC Corporation, Philadelphia, PA) at 196 g AI/ha, lambda-cyhalothrin (Karate, Syngenta Crop Protection LLC, Greensboro, NC) at 134 ml AI/ha, and thiamethoxam (Centric 40 WG, Syngenta Crop Protection, LLC, Greensboro, NC) at 175 g AI/ha. The treatments were selected based on the following discriminating action on biological control agents; (1) conservation of natural enemies (no insecticide applied); (2) natural enemies suppressed through the application of low rates of pyrethroids (lambda-cyhalothrin); (3) minimal biological control using non-selective, persistent (~10 days) insecticide (acephate); (4) partial biological control using non-selective, non-persistent (~5days) insecticide (thiamethoxam); and (5) biological control attained with a highly selective, persistent (~10 days) (flonicamid). The insecticides were applied using a $CO_2$ pressurized backpack sprayer fitted with Teejet 8006 flat spray nozzles that dispensed 14.0 ml/$m^2$ of insecticide.


Figure 3.1. Study sites in Louisiana

3.1.3. Adult stink bug sampling.

Sampling of adult stink bugs began at pod formation (R3) and ended at beginning maturity (R7) (Fehr et al. 1971). Stink bugs were collected with a 38 cm diameter sweep net on two adjacent rows per plot once a week to obtain 25 sweep samples. The rows were alternated every week to avoid the same rows being sampled twice within a period of two weeks. Samples of stink bugs were identified to species, counted, and released into the already sampled rows.

3.1.4. Natural enemy sampling.

Sampling of red imported fire ants and stink bug egg parasitoids began at the R3 soybean stage prior to insecticide treatment. Sampling continued after application of insecticides up to R7 soybean stage (Fehr et al. 1971). Red imported fire ants were collected every other week and alternated with stink bug egg parasitoid sampling in 2012 and 2013. To collect red imported fire ants,~0.125 $cm^3$ cubes of hot dogs (Bar-S, Phoenix, AZ) (bait) were placed individually in 20 ml scintillation vials (Wheaton®, Millville, NJ) (Bao et al 2011). The opened vials containing bait were placed at the center of one row per plot and left for 45 minutes (rows were alternated every other week). After the 45 minute time interval, each vial was collected and capped with a lid. The vials were placed in a freezer for one day. Thereafter, red imported fire ants from each vial were counted and recorded.

Five soybean plants were destructively sampled from two adjacent rows in each plot to collect egg parasitoids (rows were alternated every other week). Eggs per cluster were counted and each cluster was identified to species as described by Bundy and McPherson (2000). Egg clusters of *N. viridula* and *C. hilaris* were grouped and hereafter called "green complex" and all *Euschistus* spp. were categorized as "brown complex" (Temple 2011). Stink bug egg clusters were placed individually in 5 cm clear plastic containers (Wide-mouth jars; Uline, Pleasant Prairie, WI) that contained folded moistened grade one 9 cm sheet of cellulose filter paper (Whatman Inc. Sanford, ME). All egg clusters were reared in an environmental chamber (model I-36VL, Percival Scientific, Perry, IA) at 25°C, 60% RH and 14:10 (L:D) h photoperiod. Egg clusters were examined daily for stink bug nymph or parasitoid emergence. Parasitoids from the same egg cluster were placed in 95% ethyl alcohol for preservation. Afterwards, individual parasitoids were air dried and mounted as described by Johnson (1984b). Parasitoids were identified with taxonomic keys (Masner 1980, Johnson 1984a, 1984b, 1985b, 1987). Percentage of egg parasitism was calculated as [(number of parasitized eggs / number of eggs per cluster) *100].

3.1.5. Soybean seed sampling**.**

In 2011 and 2012, two center rows from each plot were harvested and yield was recorded. The 2-row wide sub-plots were harvested in 2013. Harvested soybean seeds were dried to 13% moisture in a drying oven. Thereafter, three replicates of 100 seeds were randomly collected from each plot. Each 100-seed replicate was weighed and percentage of damaged seeds due to stink bug feeding was determined according to Miller et al. (1977).

3.1.6. Data Analysis**.**

Infestation by stink bugs was estimated by calculating the cumulative insect days (CID). CID was obtained by summing up the stink bug insect-days in sequence (Rupple 1983). Abundance of red imported fire ants were also estimated by CID. CID of stink bugs, percentage of seed damage, 100-seed weight, yield, CID of red imported fire ants and percentage of egg parasitism were compared between cultivars and insecticides using two-way analysis of variance (ANOVA) in PROC MIXED with soybean cultivar as the main factor (SAS Institute 2013). Means were separated by Tukey's honestly significant difference (HSD) test with $P = 0.05$ (SAS Institute 2013). Differences between insecticide treatments were compared by one-way ANOVA in PROC MIXED and means were separated with Tukey's HSD test at $\alpha = 0.05$ (SAS Institute 2013). Analysis of egg parasitoids was only performed in 2013 on Pioneer 93Y92, Pioneer 95Y20, Pioneer 95Y40, and PR 4906 due to a small sample size.

**3.3. Results**

There were no significant differences in redbanded stink infestation between DP 4888 and PR 4906 ($F = 3.2$; df = 1; $P = 0.0761$) in 2011 (Table 3.1). Infestation by brown complex did not differ significantly between the two cultivars ($F = 1.2$; df = 1; $P = 0.1818$). DP 4888 and PR 4906 did not show significant differences in CID of green complex ($F = 1.0$; df = 1; $P = 0.3968$).

Table 3.1. Mean cumulative insect days (CID) (± SE) on soybean cultivars in 2011, 2012, and 2013

| Year | Cultivar | Brown complex | Green complex | Redbanded stink bug |
|---|---|---|---|---|
| 2011 | DP 4888 | 24.8 ± 2.5a | 19.6 ± 3.2a | 608.5 ± 67.2a |
| | PR 4906 | 19.7 ± 2.8a | 15.2 ± 2.6a | 810.8 ± 90.4a |
| 2012 | Pioneer 95Y20 | 28.3 ± 4.7b | 6.3 ± 1.4b | 58.4 ± 8.3b |
| | PR 4906 | 52.9 ± 8.7a | 13.2 ± 2.4a | 159.5 ± 14.3a |
| 2013 | AG 5332 | 11.6 ± 2.0ab | 9.8 ± 2.0ab | 23.2 ± 2.3a |
| | DP 4888 | 12.0 ± 2.0ab | 8.7 ± 1.7ab | 16.1 ± 2.0ab |
| | Pioneer 93Y92 | 15.9 ± 2.3a | 13.1 ± 1.9a | 10.9 ± 1.5b |
| | Pioneer 95Y20 | 5.6 ± 1.3b | 4.4 ± 1.4b | 9.1 ± 1.4b |
| | Pioneer 95Y40 | 12.4 ± 2.0ab | 8.4 ± 1.6ab | 12.0 ± 1.3b |
| | PR 4906 | 9.7 ± 2.1ab | 6.5 ± 1.9ab | 19.57 ± 2.2a |

Means with the same letters within a column are not significantly different (P > 0.05; Tukey's HSD) among cultivars within the same year

There was no significant cultivar effect on insecticides for redbanded stink bug ($F$ = 1.2; df = 1, 4; $P$ = 0.1818), brown complex ($F$ = 0.7; df = 1, 4; $P$ = 0.6046), and green complex infestations ($F$ = 1.4; df = 1, 4; $P$ = 0.7766) in 2012. However, Pioneer 95Y20 significantly reduced redbanded stink bug infestation ($F$ = 30.7; df = 1; $P$ < 0.0001) (Table 3.1). Brown complex infestation was also significantly lower on Pioneer 95Y20 ($F$ = 5.0; df = 1; $P$ = 0.0289). Green complex was reduced on Pioneer 95Y20 ($F$ = 3.9; df = 1; $P$ = 0.0496).

Interactions between soybean cultivars and insecticides were not significant on redbanded stink bug ($F$ = 0.4; df = 5, 20; $P$ = 0.9906), brown complex ($F$ = 0.8; df = 5, 20; $P$ = 0.7278), and green complex infestations ($F$ = 1.0; df = 5, 20; $P$ = 0.4754) in 2013. Pioneer 95Y20 significantly reduced redbanded stink bug ($F$ = 8.7; df = 5; $P$ < 0.0001) (Table 3.1). Infestation by brown complex was also significantly reduced by Pioneer 95Y20 ($F$ = 3.1; df = 5; $P$ =

0.0130). Green complex infestation on Pioneer 95Y20 was significantly lower ($F$ = 3.0; df = 5; $P$ = 0.0122) (Table 3.1).

There were no significant differences in redbanded stink bug infestations among insecticides applied on Pioneer 95Y20 ($F$ = 0.5; df = 4; $P$ = 0.7269) in 2012 (Figure 3.2a). However, redbanded stink bug infestation on PR 4906 were significantly reduced by thiamethoxam ($F$ = 9.9; df = 4; $P$ = 0.0002) in 2012 (Figure 3.2b). Brown complex was not significantly different among insecticide treatments on Pioneer 95Y20 ($F$ = 1.1; df = 4; $P$ = 0.3823) and PR 4906 ($F$ = 0.2; df = 4; $P$ = 0.9423) (Figure 3.2a; 3.2b). Green complex was also not significantly reduced by insecticides applied on either Pioneer 95Y20 ($F$ = 0.4; df = 4; $P$ = 0.8065) or PR 4906 ($F$ = 0.6; df = 4; $P$ = 0.6554) (Figure 3.2a; 3.2b).

The insecticides acephate, lambda-cyhalothrin and thiamethoxam significantly reduced brown complex on AG 5332 ($F$ = 6.1; df = 4; $P$ = 0.0008), DP 4888 ($F$ = 3.3; df = 4; $P$ = 0.0020), and PR 4906 ($F$ = 2.7; df = 4; $P$ = 0.0448) in 2013 (Figure 3.3a; 3.3b; Figure 3.5b). Redbanded stink bug and green complex infestations were not significantly impacted by insecticides applied to Pioneer 93Y92, Pioneer 95Y20, and Pioneer 95Y40 ($P$ > 0.05) (Figure 3.4a; 3.4b; 3.5a).

Stink bug damage on PR 4906 seeds was significantly higher ($F$ = 111.6; df = 1; $P$ < 0.0001) in 2011 (Table 3.2). 100-seed weights were significantly lower on PR 4906 ($F$ = 212.0; df = 1; $P$ <0.0001). However, yield was not impacted by cultivar ($F$ = 2.4; df = 1; $P$ = 0.1265) (Table 3.2). There was no significant cultivar effect on insecticide for seed damage ($F$ = 0.5; df = 1, 4; $P$ = 0.7082) in 2012. PR 4906 seeds were significantly damaged compared to Pioneer 95Y20 ($F$ = 25.7; df = 1; $P$ < 0.0001) (Table 3.2). There was a significant main factor effect on insecticide for 100-seed weight ($F$ = 3.3; df = 1, 4; $P$ = 0.0129) in 2012.

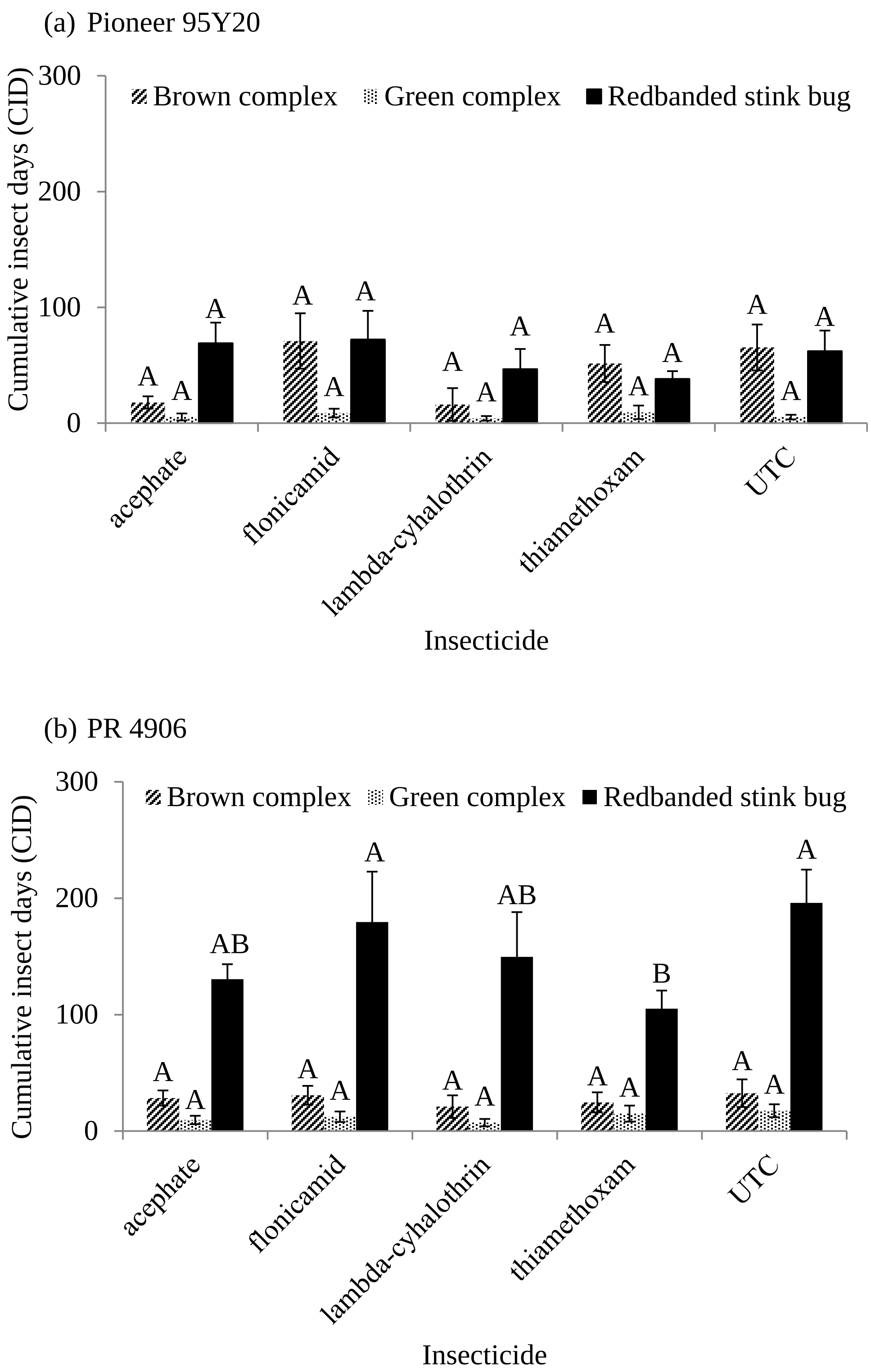
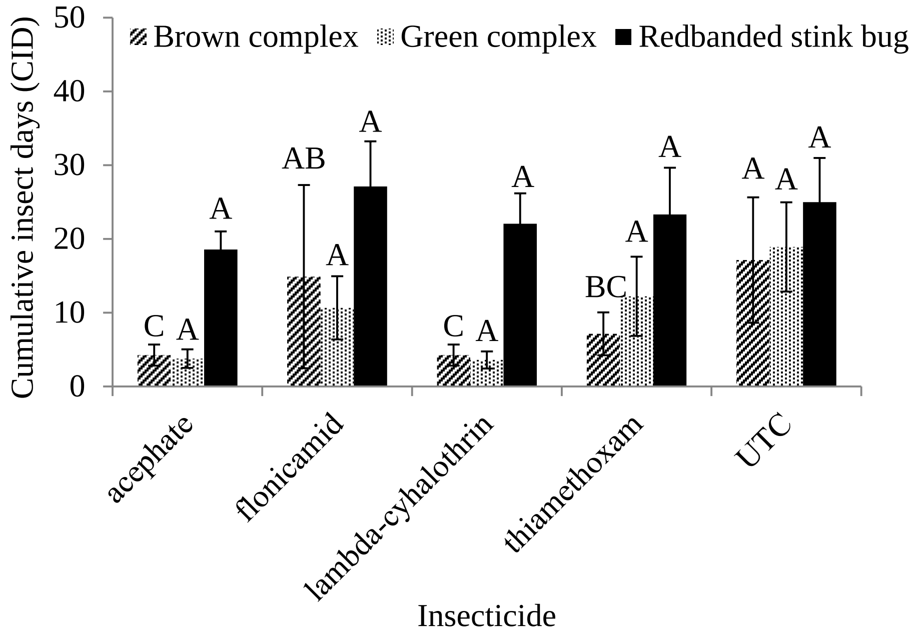



Figure 3.2. Cumulative insect days (CID) of brown complex, green complex, and redbanded stink bugs on (a) Pioneer 95Y20 and; (b) PR 4906 in 2012. Means accompanied by different letters indicate a significant difference ($P < 0.05$) within a stink bug species.

(a) AG 5332




(b) DP 4888

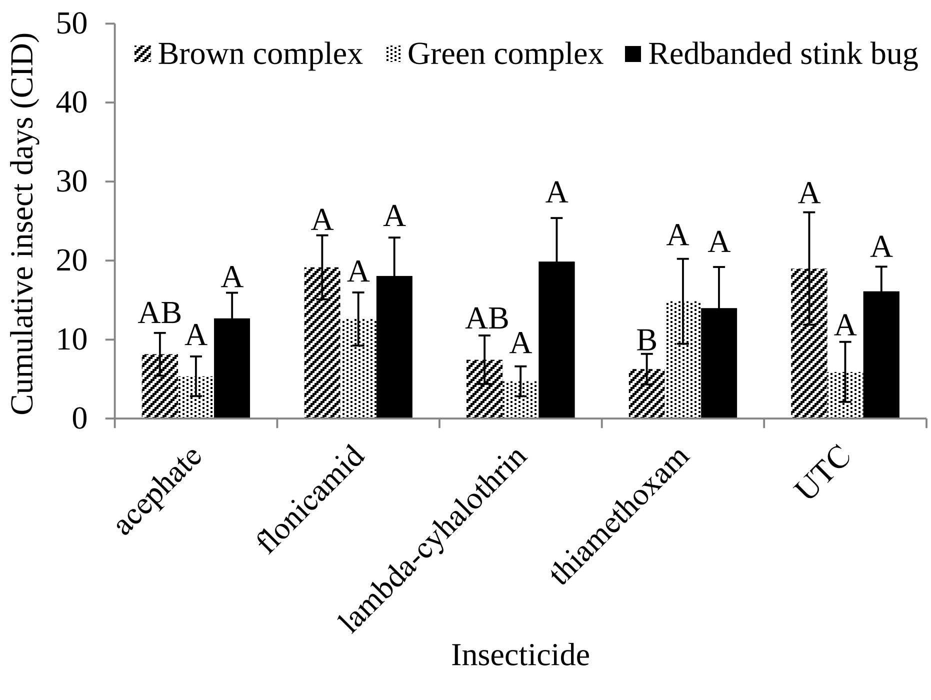
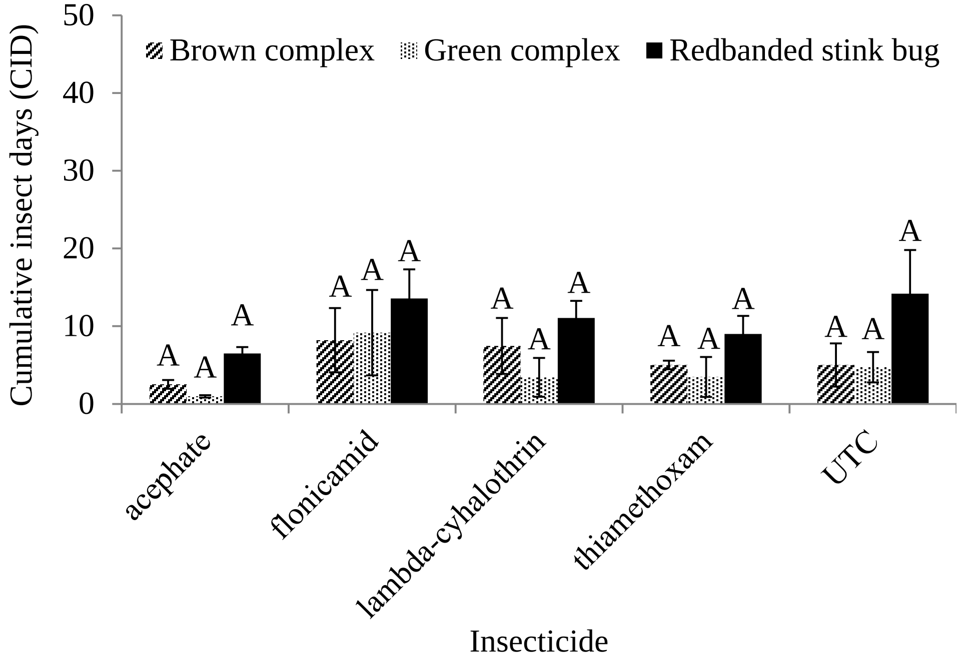



Figure 3.3. Cumulative insect days (CID) of brown complex, green complex, and redbanded stink bugs on (a) AG 5332 and; (b) DP 4888 in 2013. Means accompanied by different letters indicate a significant difference ($P < 0.05$) within a stink bug species.

a) Pioneer 93Y92




(c) Pioneer 95Y20

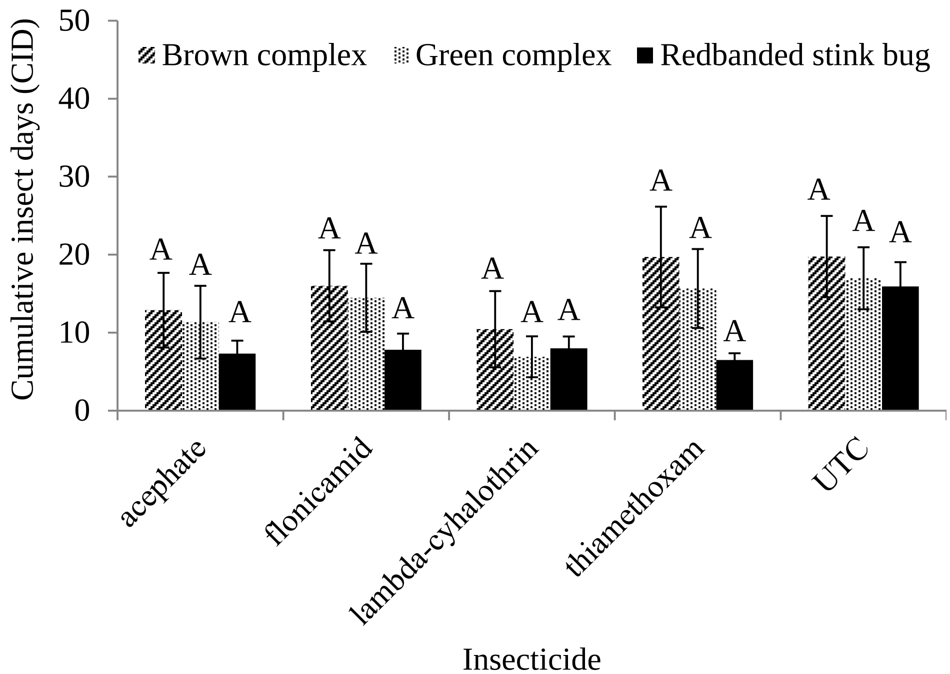
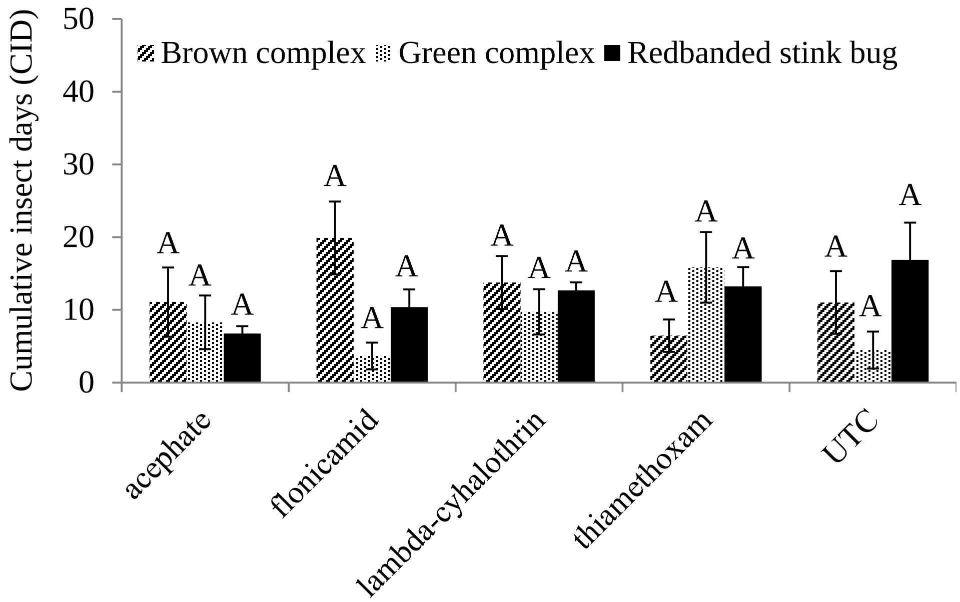



Figure 3.4. Cumulative insect days (CID) of brown complex, green complex, and redbanded stink bugs on (a) Pioneer 93Y92 and; (b) Pioneer 95Y20 in 2013. Means accompanied by different letters indicate a significant difference ($P < 0.05$) within a stink bug species.

(a) Pioneer 95Y40




(b) PR 4906

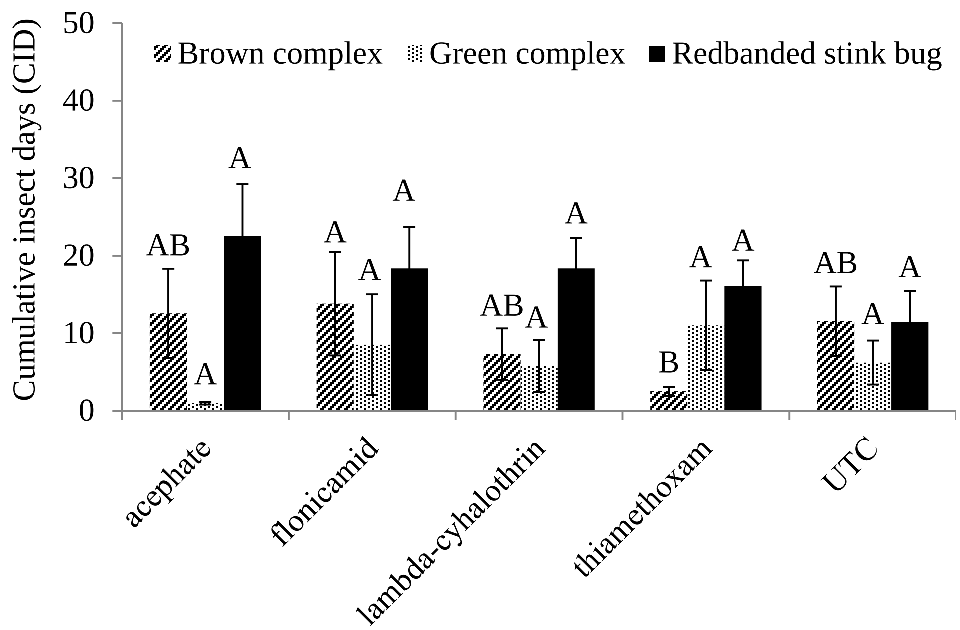



Figure 3.5. Cumulative insect days (CID) of brown complex, green complex, and redbanded stink bugs on (a) Pioneer 95Y40 and; (b) PR 4906 in 2013. Means accompanied by different letters indicate a significant difference ($P < 0.05$) within a stink bug species.

PR 4906 had significantly heavier 100-seed weight ($F = 24.0$; df = 1; $P < 0.0001$) (Table 3.2). Interactions between cultivar and insecticides were not significant for yield ($F = 0.1$; df = 1, 4; $P = 0.9939$) in 2012. However, yield from Pioneer 95Y20 was significantly higher than PR 4906 ($F = 114.9$; df = 1; $P < 0.0001$) (Table 3.2).

Table 3.2. Mean seed damage (%), 100-seed weight (g), and yield (bushels/A) (± SE) on soybean cultivars in 2011, 2012, and 2013

| Year | Cultivar | Seed damage (%) | 100-seed wt (g) | Yield (Bushels/A) |
|---|---|---|---|---|
| 2011 | DP 4888 | 45.6 ± 0.8b | 14.0 ± 0.1a | 150.1 ± 1.8a |
| | PR 4906 | 57.6 ± 0.8a | 12.4 ± 0.1b | 154.2 ± 1.9a |
| 2012 | Pioneer 95Y20 | 30.4 ± 1.6b | 13.1 ± 0.1b | 92.5 ± 2.8a |
| | PR 4906 | 59.9 ± 1.1a | 13.9 ± 0.1a | 44.3 ± 3.1b |
| 2013 | AG 5332 | 10.4 ± 0.5c | 14.9 ± 0.1bc | 93.5 ± 2.5ab |
| | DP 4888 | 12.3 ± 0.5c | 14.7 ± 0.1c | 83.4 ± 1.9bc |
| | Pioneer 93Y92 | 20.1± 0.6a | 15.9 ± 0.1a | 73.8 ± 2.0cd |
| | Pioneer 95Y20 | 5.6 ± 0.3d | 14.1 ± 0.1d | 98.0 ± 1.5a |
| | Pioneer 95Y40 | 7.2 ± 0.4d | 15.8 ± 0.1a | 86.2 ± 4.0abc |
| | PR 4906 | 18.1 ± 0.7b | 15.1 ± 0.1b | 61.6 ± 5.4d |

Means with the same letters within a column are not significantly different ($P > 0.05$; Tukey's HSD) among cultivars within the same year

There were no significant differences in seed damage ($F = 1.0$; df = 1; $P = 0.4160$), 100-seed weight ($F = 0.9$; df = 1; $P = 0.6054$), and yield ($F = 0.1$; df = 1; $P = 0.9934$) among insecticides applied to Pioneer 95Y20 in 2012 (Table 3.3). In contrast, thiamethoxam significantly reduced seed damage on PR 4906 ($F = 2.9$; df = 1; $P = 0.0322$) (Table 3.3). Application of thiamethoxam also significantly increased 100-seed weight on PR 4906 in 2012 ($F = 4.0$; df = 4; $P = 0.\ 0048$).

However, yield was not significantly different among insecticide treatments on PR 4906 ($F$ = 0.2; df = 1; $P$ = 0.9495).

Table 3.3. Mean seed damage (%), 100-seed weight (g), and yield (bushels/A) (± SE) among insecticides applied to Pioneer 95Y20 and PR 4906 in 2012

| Cultivar | Insecticide | Seed damage (%) | 100-seed wt (g) | Yield (Bushels/A) |
|---|---|---|---|---|
| Pioneer 95Y20 | acephate | 27.3 ± 2.6a | 13.4 ± 0.3a | 93.5 ± 6.9a |
| | flonicamid | 22.5 ± 2.0a | 13.1 ± 0.3a | 94.3 ± 8.1a |
| | lambda-cyhalothrin | 21.4 ± 2.5a | 13.6 ± 0.3a | 92.8 ± 7.1a |
| | thiamethoxam | 22.3 ± 2.2a | 12.7 ± 0.3a | 93.4 ± 8.5a |
| | UTC | 25.5 ± 3.0a | 13.3 ± 0.3a | 90.6 ± 4.5a |
| PR 4906 | acephate | 54.2 ± 2.9ab | 13.5 ± 0.2b | 44.8 ± 6.8a |
| | flonicamid | 51.8 ± 2.4ab | 14.1 ± 0.1ab | 46.2 ± 8.4a |
| | lambda-cyhalothrin | 54.2 ± 1.7ab | 13.9 ± 0.2ab | 41.3 ± 7.9a |
| | thiamethoxam | 49.9 ± 2.5b | 14.5 ± 0.2a | 49.0 ± 9.4a |
| | UTC | 58.8 ± 1.1a | 13.5 ± 0.2b | 42.3 ± 4.9a |

Means with the same letters within a column are not significantly different (P > 0.05; Tukey's HSD) among insecticides within each cultivar

Interactions between cultivars and insecticides were significant for seed damage in 2013 ($F$ = 2.0; df = 5, 20; $P$ = 0.0056). However, Pioneer 95Y20 provided significant protection against seed damage compared to Pioneer 93Y92 ($F$ = 142.0; df = 5; $P$ < 0.0001) (Table 3.2). There was no significant cultivar effect on insecticides for 100-seed weight in 2013 ($F$ = 0.5; df = 5, 20; $P$ = 0.9592). 100-seed weight were significantly higher on Pioneer 93Y92 ($F$ = 63.5; df = 5; $P$ < 0.0001) (Table 3.2). Main factor effect on insecticide treatments was not significant for yield in 2013 ($F$ = 0.3; df = 1, 4; $P$ = 0.9996). Nevertheless, cultivar significantly affected yield ($F$ = 16.2; df = 5; $P$ < 0.0001) with the highest yield from Pioneer 95Y20 in 2013 (Table 3.2).

Treatment with thiamethoxam on DP 4888, Pioneer 93Y92, and PR 4906 led to significantly less stink bug damage in 2013 (Table 3.4). Treatment with insecticides on all cultivars had no significant impact on 100-seed weight in 2013 (Table 3.5). Yield was similar among insecticides applied to all six cultivars in 2013 (Table 3.6).

Insecticide treatments did not have a significant interaction with either Pioneer 95Y20 ($F$ = 1.20; df = 1, 4; $P$ = 0.2417) or PR 4906 ($F$=0.78; df = 1, 4; $P$ = 0.7645) on red imported fire ants in 2012. Likewise, abundance of red imported fire ants was not significantly enhanced on either soybean cultivar ($F$ = 7.43; df = 1; $P$ = 0.096) (Fig. 3.6). In 2013, cultivars did not have an effect on insecticide application on the CID of fire ants ($F$ = 0.4; df = 5, 20; $P$ = 0.9899). However, Pioneer 95Y40 significantly increased CID of red imported fire ants ($F$ = 5.5; df = 5; $P$ = 0.0002) (Figure 3.7). CID were not significantly different among insecticides on AG 5332 ($F$ = 0.2; df = 4; $P$ = 0.9105), DP 4888 ($F$ = 1.1; df = 4; $P$ = 0.3914), Pioneer 93Y92 ($F$ = 0.9; df = 4; $P$ = 0.4971), Pioneer 95Y20 ($F$ = 0.3; df = 4; $P$ = 0.9044), Pioneer 95Y40 ($F$ = 0.6; df = 4; $P$ = 0.6928), and PR 4906 ($F$ = 0.4; df = 4; $P$ = 0.8150).

Redbanded stink bug eggs made up 66.5 ± 16.7% of the stink bug eggs collected and were significantly higher than brown complex at 4.8 ± 3.7%, and green complex at 14.8 ± 11.4% in 2013 ($F$ = 7.8; df = 2; $P$ = 0.0048). *Telenomus podisi* Ashmead (Hymenoptera: Plastygastridae) parasitized 32.3 % of the eggs and 14.6 % were parasitized by *Trissolcus euschisti* (Ashmead) (Hymenoptera: Plastygastridae). Soybean cultivar had no significant effect on insecticide for egg parasitism ($F$ = 0.8; df = 5, 20; $P$ = 0.7032) and egg parasitism did not vary between cultivars ($F$ = 0.4; df = 5; $P$ = 0.8352). Insecticides had no significant impact on egg parasitism on Pioneer 93Y92 ($F$ = 1.0; df = 4; $P$ = 0.4406), Pioneer 95Y20 ($F$ = 1.1; df = 4; $P$ = 0.3895), Pioneer 95Y40 ($F$ = 1.0; df = 4; $P$ = 0.4406), and PR 4906 ($F$ = 1.0; df = 4; $P$ = 0.4106) (Table 3.7).

Table 3.4. Mean seed damage (%) (± SE) on insecticides applied on soybean cultivars in 2013

| Insecticide | AG 5332 | DP 4888 | Pioneer 93Y92 | Pioneer 95Y20 | Pioneer 95Y40 | PR 4906 |
|---|---|---|---|---|---|---|
| acephate | 10.0 ± 0.8ab | 11.5 ± 0.7ab | 22.5 ± 1.6a | 5.3 ± 0.6a | 6.3 ± 0.6a | 16.6 ± 1.5b |
| flonicamid | 10.5 ± 0.9ab | 14.4 ± 1.1a | 21.0 ± 1.1ab | 6.3 ± 0.5a | 9.42 ± 0.9a | 19.6 ± 1.8ab |
| lambda-cyhalothrin | 10.3 ± 1.2ab | 12.8 ± 1.2ab | 20.7 ± 1.7ab | 5.8 ± 0.8a | 6.9 ± 0.7a | 16.9 ± 1.5b |
| thiamethoxam | 8.5 ± 1.1b | 9.8 ± 0.7b | 16.5 ± 1.2b | 5.0 ± 0.4a | 6.5 ± 0.9a | 14.1 ± 1.8b |
| UTC | 12.7 ± 1.1a | 12.9 ± 1.0ab | 19.9 ± 1.3ab | 5.8 ± 0.7a | 6.6 ± 0.9a | 23.2 ± 1.7a |
| df | 4 | 4 | 4 | 4 | 4 | 4 |
| *F* | 3.1 | 3.2 | 2.6 | 0.7 | 2.6 | 5.3 |
| *P* | 0.0182 | 0.0155 | 0.0407 | 0.6024 | 0.0464 | 0.0006 |

Means with the same letters within a column are not significantly different (P > 0.05; Tukey's HSD)

Table 3.5. Mean of 100-seed weight (g) (± SE) on insecticides applied on soybean cultivars in 2013

| Insecticide | AG 5332 | DP 4888 | Pioneer 93Y92 | Pioneer 95Y20 | Pioneer 95Y40 | PR 4906 |
|---|---|---|---|---|---|---|
| acephate | 15.0 ± 0.2a | 14.8 ± 0.1a | 15.8 ± 0.2a | 14.3 ± 0.2 | 15.9 ± 0.1a | 15.1 ± 0.2a |
| flonicamid | 14.7 ± 0.3a | 14.6 ± 0.2a | 15.8 ± 0.2a | 14.0 ± 0.2a | 15.5 ± 0.2a | 15.0 ± 0.2a |
| lambda-cyhalothrin | 14.9 ± 0.3a | 14.5 ± 0.2a | 16.0 ± 0.2a | 14.2 ± 0.2a | 15.7 ± 0.1a | 15.0 ± 0.3a |
| thiamethoxam | 14.9 ± 0.2a | 14.8 ± 0.1a | 16.0 ± 0.2a | 14.1 ± 0.1a | 15.7 ± 0.1a | 15.4 ± 0.2a |
| UTC | 14.7 ± 0.3a | 14.6 ± 0.1a | 15.8 ± 0.2a | 13.8 ± 0.1a | 16.0 ± 0.1a | 15.0 ± 0.2a |
| df | 4 | 4 | 4 | 4 | 4 | 4 |
| *F* | 0.2 | 0.7 | 0.22 | 1.5 | 2.0 | 0.7 |
| *P* | 0.9302 | 0.5725 | 0.9287 | 0.2195 | 0.1073 | 0.5879 |

Means with the same letters within a column are not significantly different (P > 0.05; Tukey's HSD)

Table 3.6. Mean yield (bushels/A) (± SE) on insecticides applied on soybean cultivars in 2013

| Insecticide | AG 5332 | DP 4888 | Pioneer 93Y92 | Pioneer 95Y20 | Pioneer 95Y40 | PR 4906 |
|---|---|---|---|---|---|---|
| acephate | 94.5 ± 5.1a | 78.0 ± 3.9a | 74.0 ± 3.7a | 95.6 ± 4.7a | 87.9 ± 10.4a | 60.5 ± 12.4a |
| flonicamid | 94.3 ± 7.2a | 83.6 ± 3.4a | 71.1 ± 4.6a | 96.9 ± 2.1a | 81.8 ± 10.8a | 58.5 ± 13.0a |
| lambda-cyhalothrin | 98.5 ± 3.9a | 88.7 ± 5.2a | 68.5 ± 6.6a | 101.7 ± 2.39a | 84.2 ± 8.9a | 60.6 ± 10.8a |
| thiamethoxam | 92.7 ± 6.3a | 87.1 ± 2.7a | 77.6 ± 3.3a | 96.0 ± 4.0a | 91.1 ± 8.0a | 63.1 ± 12.4a |
| UTC | 87.5 ± 5.8a | 79.3 ± 5.0a | 77.9 ± 2.7a | 100.16 ± 3.6a | 86.0 ± 8.5a | 65.2 ± 14.3a |
| df | 4 | 4 | 4 | 4 | 4 | 4 |
| *F* | 0.5 | 1.3 | 0.9 | 0.6 | 0.1 | 0.0 |
| *P* | 0.7512 | 0.2976 | 0.4870 | 0.6687 | 0.9659 | 0.9963 |

Means with the same letters within a column are not significantly different (P > 0.05; Tukey's HSD)

Table 3.7. Mean percentage of egg parasitism (%) (± SE) on insecticides applied on Pioneer 93Y92, Pioneer 95Y20, Pioneer 95Y40, and PR 4906 in 2013

| Insecticide | Pioneer 93Y92 | Pioneer 95Y20 | Pioneer 95Y40 | PR 4906 |
|---|---|---|---|---|
| acephate | 0.0 ± 0.0a | 0.0 ± 0.0a | 0.0 ± 0.0a | 0.0 ± 0.0a |
| flonicamid | 0.0 ± 0.0a | 0.0 ± 0.0a | 0.0 ± 0.0a | 0.0 ± 0.0a |
| lambda-cyhalothrin | 0.0 ± 0.0a | 0.0 ± 0.0a | 0.0 ± 0.0a | 0.0 ± 0.0a |
| thiamethoxam | 0.0 ± 0.0a | 0.0 ± 0.0a | 3.7 ± 1.6a | 0.0 ± 0.0a |
| UTC | 4.0 ± 2.0a | 3.5 ± 1.5a | 0.0 ± 0.0a | 2.4 ± 1.0a |

Means with the same letters within a column are not significantly different (P > 0.05; Tukey's HSD)

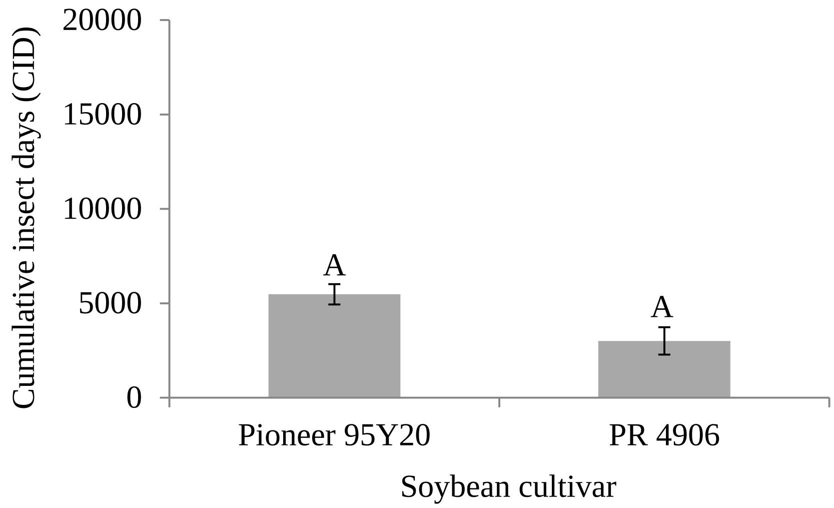



Figure 3.6. Cumulative insect days (CID) of red imported fire ants from Pioneer 95Y20 and PR 4906 in 2012. Means accompanied by different letters indicate a significant difference ($P < 0.05$).

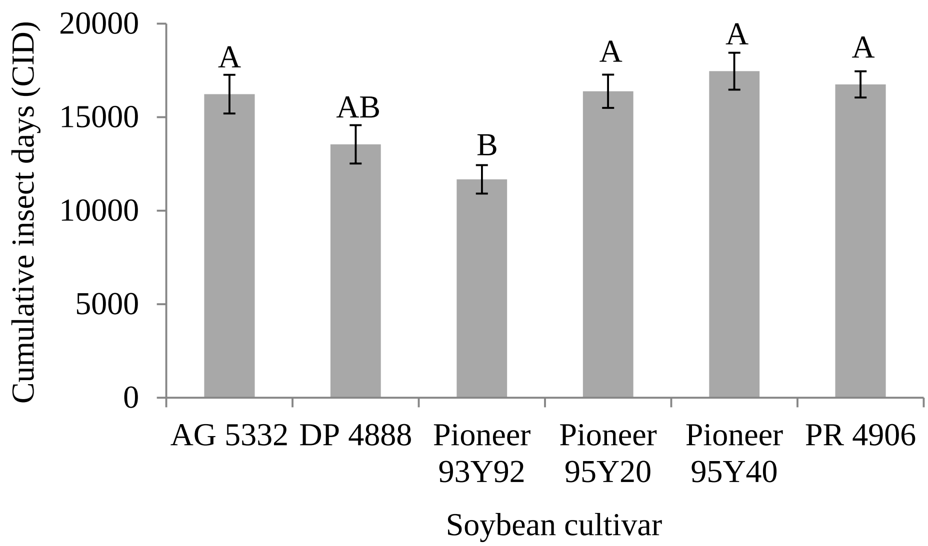



Figure 3.7. Cumulative insect days (CID) of red imported fire ants from AG 5332, DP 4888, Pioneer 93Y92, Pioneer 95Y20, Pioneer 95Y40, and PR 4906 in 2013. Means accompanied by different letters indicate a significant difference ($P < 0.05$).

### 3.4. Discussion

The results from this study demonstrated that plant resistance plays a major role in negatively impacting the pest pressure of stink bugs than insecticide application. The consistently low CID on Pioneer 95Y20 was an indication that stink bug numbers were reduced. From these results, it is possible that non-preference was the mechanism of resistance for Pioneer 95Y20. McPherson et al. (2007) proposed that soybean cultivars with low stink bug infestations demonstrated non-preference. Insect resistant soybean cultivars have been also been reported to be have less seed damage (Campos et al. 2010). Accordingly, Pioneer 95Y20 showed some form of resistance to stink bugs by having the least amount of seed damage. However, this cultivar did not positively impact 100-seed weight. The 100-seed weight ranged from 12–16.1 g, consistent with cultivars evaluated by McPherson et al. (2007). Nevertheless, overall yield from Pioneer 95Y20 was higher than other cultivars and amounted to~100 bushels/A. Higher yields have also been previously demonstrated in moderately insect resistant soybean cultivars (McPherson et al 2007). PR 4906 showed susceptibility to stink bug infestation, and had higher damage and low yield throughout the study.

Apparently, insecticides were not as effective as resistant soybean cultivars in reducing stink bug pest pressure in the present study. At present, redbanded stink bug is the predominant phytophagous pentatomid species in Louisiana and tolerance to major classes of insecticides by this pest was reported (Baur et al. 2010, Temple et al. 2013b). Nevertheless, thiamethoxam significantly reduced the number of brown complex and redbanded stink bugs when contrasted to other insecticides. Baur et al. (2000) reported that thiamethoxam has the potential to provide control exceeding 70 %. The present study also showed that seed damage was reduced by application of thiamethoxam and to a lesser extent, 100-seed weight was increased by this insecticide. On the contrary, yield was not impacted by the application of any insecticide.

This current study also demonstrated that soybean cultivars can have a positive effect on the number of fire ants. However, CID of red imported fire ants were similar between moderately resistant soybean cv. Pioneer 95Y20 and susceptible cv. PR 4906 in 2013. Not much is known about the association of red imported fire ants and resistant soybean cultivars. However, it was reported that insect resistant soybean cultivars can attract a higher number of natural enemies (Michereff et al. 2014).

Generally, insecticides did not enhance egg parasitism in this study and it is possible that parasitoid emergence was precluded. 8-day old insecticide residues on plants were found to be toxic to stink bug egg parasitoids (Smilanick et al. 1995). Koppel et al. (2011) reported mortality of 100% mortality of *T. podisi* in field experiments caused by lambda-cyhalothrin. They also demonstrated that some insecticides were more toxic to developing *T. podisi* than to developing brown stink bug. Other insecticides know to be toxic to stink bug egg parasitoids include acephate and thiamethoxam (Koppel et al. 2011, Turchen et al. 2015). The non-persistent or selective insecticides in the present study may have had similar adverse effects on egg parasitoids and thus, prevent their emergence.

The present study demonstrated that a combination of insect resistant soybean cultivars with non-persistent insecticides may provide appreciable amount of stink bug control on soybeans. In addition, resistant soybean cultivars were shown to have a positive impact on the red imported fire ants which can enhance mortality of stink bugs. Overall, this study demonstrated that plant resistance was an important factor in the management of stink bugs on soybeans because the moderately resistant Pioneer 95Y20 had consistently reduced pest pressure, less seed damage, and high yields. To some degree, the insecticide thiamethoxam also provided better control than the other selected insecticides. This was an interesting outcome because thiamethoxam is not among the recommended insecticides for the control of stink bugs on commercial soybeans in Louisiana. However, one of the recommended insecticides is a mixture of lambda-cyhalothrin and thiamethoxam (Endigo ZC, Syngenta Crop Protection, LLC, Greensboro, NC) (Davis 2014). In conclusion, growers should plant insect resistance soybean cultivars as an integral component of stink bug management. The continued use of insecticides when needed would further mitigate any shortfalls of the resistant soybean cultivars.

# CHAPTER 4: IMPACT OF EXOGENOUS METHYL JASMONATE ON ATTRACTION OF NATURAL ENEMIES OF STINK BUGS (HEMIPTERA: PENTATOMIDAE) ON SOYBEANS

## 4.1. Introduction

Stink bugs (Hemiptera: Pentatomidae) cause considerable damage to soybeans, *Glycine max* (L.) Merrrill (Russin et al. 1987, Corrêa-Ferreira and de Azevedo 2002), manifested as underdeveloped pods (Corrêa-Ferreira and de Azevedo 2002) that can lead to reduced yield and poor seed quality (McPherson et al. 1994). To lessen these damaging effects on soybeans, economic thresholds, and economic injury levels have been established (Musser et al. 2011). Nevertheless, naturally occurring natural enemies can cause stink bug mortality (Orr et al. 1986, Koppel et al. 2009). Several studies have reported that insecticide application can have deleterious effects on natural enemies of stink bugs (Sudarsono et al. 1992, Smilanick et al. 1996, Koppel et al. 2011, Turchen et al. 2015). Still, chemical control and the conservation of biological control agents can be compatible (Sudarsono et al. 1992).

Attraction of biological control agents was demonstrated by exogenous application of *cis*-jasmone on soybean plants (Vieira et al. 2013). *Cis*-jasmone is a derivative of jasmonic acid (JA) (von Dahl and Baldwin 2004), and a constituent of plant volatiles that are released when herbivory occurs (Loughrin et al. 1995, Pare and Tumlinson 1997). JA is a plant growth regulating hormone and has been associated with developmental processes such as senescencing of leaves (Meyer et al. 1984, Anderson 1989). It was also reported that *cis*-jasmone induces the synthesis of herbivore induced plant volatiles (HIPVs) in soybean plants, and these compounds are similar to the naturally induced HIPVs caused by stink bug feeding and oviposition (Moraes et al. 2008).

Several studies have evaluated the attractiveness of *cis*-jasmone to natural enemies in different plants (Moraes et al. 2005, Moraes et al. 2008, Simpson et al. 2011, Vieira et al. 2013). For example, when *cis*-jasmone increased the attractiveness of parasitoids of aphid pests (Bruce et al. 2003).

Methyl jasmonate (MeJA) is another derivative of jasmonic acid (von Dahl and Baldwin 2004). In comparable studies to *cis*-jasmone, it was shown that methyl jasmonate was an attractant for parasitoids in grapevines (James and Grasswitz 2005). Rodriguez-Saona et al. (2001) further reported that synthetic methyl jasmonate mimicked the plant response induced by insect herbivory through emission of volatiles in cotton plants. However, there are few studies on the effects of methyl jasmonate (MeJA) on natural enemies of stink bugs infesting soybean plants. Therefore, it was postulated that the abundance of natural enemies in soybean would be affected by exogenous application of methyl jasmonate.  The objective of this study was to determine the effect of MeJA on the abundance of predators and stink bug egg parasitoids over sampling dates.

**4.2. Materials and Methods**

In 2014, this experiment was conducted at three Louisiana State University Agricultural Center Research Stations (LSU AgCenter) in Crowley (Rice Research Station), Jeanerette (Iberia Research Station), and St. Joseph (Northeast Research Station) (Figure 4.1). In 2015, the experiment was carried out in Alexandria (Dean Lee Research Station, Baton Rouge (Ben Hur Research Farm), Chase (Sweet Potato Research Station), Crowley (Rice Research Station), and Jeanerette (Iberia Research Station).

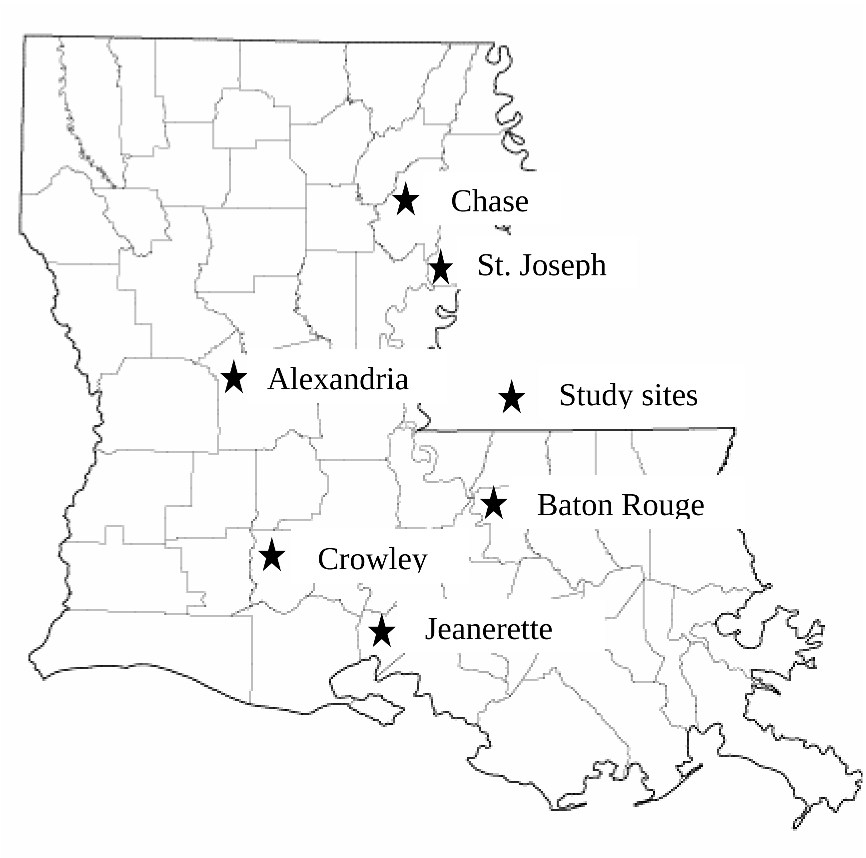



Figure 4.1. Study sites in Louisiana in 2014 and 2015

During both years, soybean varieties Asgrow AG 4034 (Monsanto, St. Louis, MO), Asgrow AG 4633 (Monsanto, St. Louis, MO), and Asgrow AG 5535 (Monsanto, St. Louis, MO) were each planted in large plots 0.1 ha at each location. However, soybean varieties Credenz™ CZ 3945 LL (Monsanto, St. Louis, MO), Credenz™ CZ 4748 LL (Monsanto, St. Louis, MO), Credenz™ CZ 5445 LL (Monsanto, St. Louis, MO) were planted in Alexandria during 2015. The recommended agronomic practices were applied to all soybean fields without insecticides (Levy et al. 2016). At each location, six adjacent plots measuring 7.6 m long by 4 rows wide were randomly selected and demarcated in each the three soybean cultivars. Out of the six plots in each cultivar, three were sprayed with methyl jasmonate (Bedoukin Research, Denbury, CO) at a concentration of 10 ppm (~ 45 ppm) (Penninckx et al. 1998). The other three plots were left untreated. The three treated plots were sprayed using a $CO_2$ pressurized backpack sprayer fitted with Teejet 8006 flat spray nozzles. Treatment with methyl jasmonate began on dates when the soybean plants reached pod development stage (R3) and a second spraying was done when plants reached pod filling (R5.5) (Fehr et al. 1971) (Table 4.1). Sampling of fire ants and parasitoids on all six plots per MG began two days after the first application of methyl jasmonate. Thereafter, sampling was conducted on a weekly basis until the second application of methyl jasmonate. Plots were also sampled two days after the second treatment and then weekly sampling resumed until physiological maturity (R8) of soybean plants.

Table 4.1. Spraying dates for MeJA in 2014 and 2015

| Year | Location | First treatment | Second treatment |
|---|---|---|---|
| 2014 | Crowley | June 23 | July 14 |
| | Jeanerette | June 30 | July 21 |
| | St. Joseph | July 23 | August 13 |
| 2015 | Alexandria | August 4 | August 26 |
| | Baton Rouge | July 21 | August 18 |
| | Chase | July 7 | August 3 |
| | Crowley | July 21 | August 12 |
| | Jeanerette | June 23 | July 19 |

4.2.1. Red imported fire ants (RIFA) sampling.

Sampling of red imported fire ants (RIFA) was started at 2 days after treatment (DAT) and thereafter, weekly. The ants were collected using ~ 0.125 $cm^3$ cubes of hot dogs (Bar-S, Phoenix, AZ) (bait) placed individually in 20 ml scintillation vials (Wheaton®, Millville, NJ) (Bao et al.

2011). Three vials containing bait were placed at three points along one row per plot and left for 5 minutes. Another set of three vials were also placed at three points along an adjacent row in the same plot and left for 45 minutes (rows were alternated during each sampling week). The time intervals were based on previous experiments that exposed predators and parasitoids to jasmonic acid treated plants for a maximum of 5 minutes (Dicke et al. 1999, Gols et al. 2003) or 45 minutes (Williams et al. 2008). After the prescribed time lapsed, each vial with fire ants was collected and capped with a lid. The collected fire ants were placed in a freezer for at least one day and then counted.

4.2.2. Parasitoid sampling.

Ten plants were randomly collected by destructive sampling on two adjacent rows from each plot and rows were alternated every week in each plot. All plant structures were visually examined for the presence of stink bug egg clusters. The number of eggs per cluster was recorded and identified to species level where possible but all egg clusters belonging to the genera *Euschistus* (Hemiptera: Pentatomidae) were grouped together according to Temple (2011). The eggs of southern green stink bug, *Nezara viridula* (L.) (Hemiptera: Pentatomidae) and green stink bug *Chinavia hilaris* Say (Hemiptera: Pentatomidae) were grouped together and hereafter called “green stink bug complex”.

The collected stink bug egg clusters (still attached to plant substrate) were placed in 5 cm clear plastic containers (Wide-mouth jars; Uline, Pleasant Prairie, WI) that contained moistened folded grade one 9 cm sheet of cellulose filter paper (Whatman Inc. Sanford, ME). These eggs were later reared in a growth chamber (model I-36VL, Percival Scientific, Perry, IA) at 25°C, 60% RH and 14:10 (L:D) h photoperiod. Egg clusters were monitored daily until stink bug nymphs hatched or parasitoids emerged. The emerged nymphs were discarded, and the parasitoids were preserved in vials containing 95% ethyl alcohol. All emerged parasitoids were air dried and mounted on triangular card points (Johnson 1984b) and identified to species level using taxonomic keys (Masner 1980, Johnson 1984a, 1984b, 1985b, 1987).

4.2.3. Data Analysis.

Repeated measures were performed (PROC GLIMMIX, SAS Institute 2013) to determine the effect of methyl jasmonate on the abundance of red imported fire ants for the two time intervals (5 and 45 minutes) at different sampling dates/times. Repeated measures using PROC GLIMMIX (SAS Institute 2013) were also performed to determine the effect of methyl jasmonate on the abundance of stink bug egg parasitoids at different sampling dates. The species composition of stink bug eggs was analyzed using one-way analysis of variance (ANOVA) (PROC GLM, SAS Institute 2013). Post hoc comparisons were made using Tukey's honestly significant difference (HSD) test at $\alpha = 0.05$ (PROC GLM, SAS Institute 2013).

**4.3. Results**

In 2014, treatment with methyl jasmonate at the 5-minute time interval did not have an effect on the number of fire ants because no significant interaction was observed ($F = 0.7$; df = 1, 6; $P = 0.6527$). Likewise, the number of fire ants in the treated and untreated plots were not significantly different ($F = 0.1$; df = 1; $P = 0.7282$). The number of fire ants among sampling dates did not differ significantly ($F = 1.8$; df = 6; $P = 0.0955$) (Table 4.2). At the 45-minute time interval, methyl jasmonate did not have an effect on the number of fire ants ($F = 0.3$; df = 1, 6; $P = 0.9331$). There were no significant differences between treated and untreated plots ($F = 1.3$; df = 1; $P = 0.2565$) but there were significantly high numbers of ants collected at 2 DAT and14 DAT ($F = 13.8$; df = 6; $P < 0.0001$) (Table 4.2).

In 2015, methyl jasmonate had an effect on the number of fire ants because a significant interaction was observed between methyl jasmonate treatment and sampling date at the 5-minute time interval ($F$ = 2.7; df = 1, 5; $P$ = 0.0312). In addition, the number of fire ants was higher in the treated plots compared to the untreated plots ($F$ = 9.7; df = 1; $P$ = 0.0229). The number of fire ants collected at 35 DAT was significantly higher ($F$ = 9.7; df = 5; $P$ < 0.0001) (Table 4.3). At the 45-minute time interval, methyl jasmonate did not have a significant effect on number of fire ants collected ($F$ = 1.2; df = 1, 5; $P$ = 0.2919). Similarly, there was no significant difference between treatments ($F$ = 3.0; df = 5; $P$ = 0.0834) and sampling dates did not have a significant effect on the number of fire ants collected ($F$ = 0.9; df = 5; $P$ = 0.5003) (Table 4.3).

A significantly high percentage of redbanded stink bug, *Piezodorus guildinii* (Westwood) (Hemiptera: Pentatomidae) eggs were collected in 2014 ($F$ = 37.1; df =2; $P$ < 0.0001 and in 2015 ($F$ = 33.7; df = 2; $P$ < 0.0001) (Table 4.4.) *Telenomus podisi* Ashmead (Hymenoptera: Platygastridae) was the only parasitoid that emerged from the parasitized eggs during the entire study.

Methyl jasmonate had no significant effect on the parasitism rates by *T. podisi* ($F$ = 1.9; df = 1, 6; $P$ = 0.1134) and no significant differences were observed between treatments in 2014 ($F$ = 0.8; df = 1; $P$ = 0.3707). However, significantly high parasitism rates were observed at 2 DAT and 14 DAT ($F$ = 2.8; df = 6; $P$ = 0.0248) (Figure 4.2). In 2015, there were no significant effects of methyl jasmonate on parasitism rates ($F$ = 0.5; df =1, 5; $P$ = 0.7855). Further, no significant differences were detected between treatments ($F$ = 0.6; df = 1; $P$ = 0.4478) and among sampling dates ($F$ = 0.4; df = 5; $P$ = 0.8623) (Figure 4.3).

Table 4.2. Mean number of red imported fire ants (± SE) at different sampling dates in 2014

| Time | Treatment | 2 DAT | 7 DAT | 14 DAT | 21 DAT | 28 DAT | 35 DAT | 42 DAT |
|---|---|---|---|---|---|---|---|---|
| 5-minutes | Treated | 4.5 ± 1.7a | 16.1 ± 3.7a | 8.9.0 ± 3.2a | 9.2 ± 3.7a | 12.5 ± 5.8a | 10.2 ± 5.9a | 0.1 ± 0.0a |
| | Untreated | 8.0 ± 2.7a | 13.2 ± 3.5a | 18.0 ± 5.5a | 6.3 ± 2.7a | 10. 8 ± 4.7a | 11.4 ± 5.5a | 0.1 ± 0.0a |
| 45-minutes | Treated | 70.9 ± 9.7a | 44.2 ± 6.0ab | 77.1 ± 9.5a | 47.7 ± 0.5ab | 22.5 ± 8.3b | 13.0 ± 4.8b | 14.2 ± 5.9b |
| | Untreated | 88.9 ± 10.8a | 48.8 ± 6.8ab | 71.9 ± 8.4a | 55.6 ± 0.5ab | 18.0 ± 5.9b | 23.0 ± 7.2b | 12.6 ± 5.0b |

Means with the same letters within a row are not significantly different (P > 0.05; Tukey’s HSD)

Table 4.3. Mean number of red imported fire ants (± SE) at different sampling dates in 2015

| Time | Treatment | 2 DAT | 7 DAT | 14 DAT | 21 DAT | 28 DAT | 35 DAT |
|---|---|---|---|---|---|---|---|
| 5-minutes | Treated | 14.2 ± 3.0b | 16.0 ± 3.0b | 28.6 ± 5.4b | 16.1 ± 4.1b | 15.5 ± 6.8b | 86.0 ± 18.1a |
| | Untreated | 9.5 ± 2.6b | 21.2 ± 4.7b | 21.5 ± 4.08b | 16.1 ± 4.0b | 11.0 ± 4.0b | 44.9 ± 12.1a |
| 45-minutes | Treated | 49.4 ± 4.8a | 35.9 ± 4.8a | 46.7 ± 5.8a | 56.6 ± 8.4a | 42.7 ± 8.5a | 47.6 ± 17.1a |
| | Untreated | 44.9 ± 4.6a | 44.8 ± 4.9a | 37.68 ± 5.0a | 41.2 ± 6.0a | 38.4 ± 7.2a | 22.7 ± 9.5a |

Means with the same letters within a row are not significantly different (P > 0.05; Tukey’s HSD)

Table 4.4. Mean percentage (%) of stink bug species (± SE) in 2014 and 2015

| Year | *Euschistus* spp. | Green stink bug complex | *P. guildinii* |
|---|---|---|---|
| 2014 | 8.4 ± 3.1c | 27.0 ± 4.9b | 64.7 ± 5.8a |
| 2015 | 14.4 ± 3.7b | 14.4 ± 6.0b | 71.0 ± 6.7a |

Means with the same letters within a row are not significantly different (P > 0.05; Tukey’s HSD)

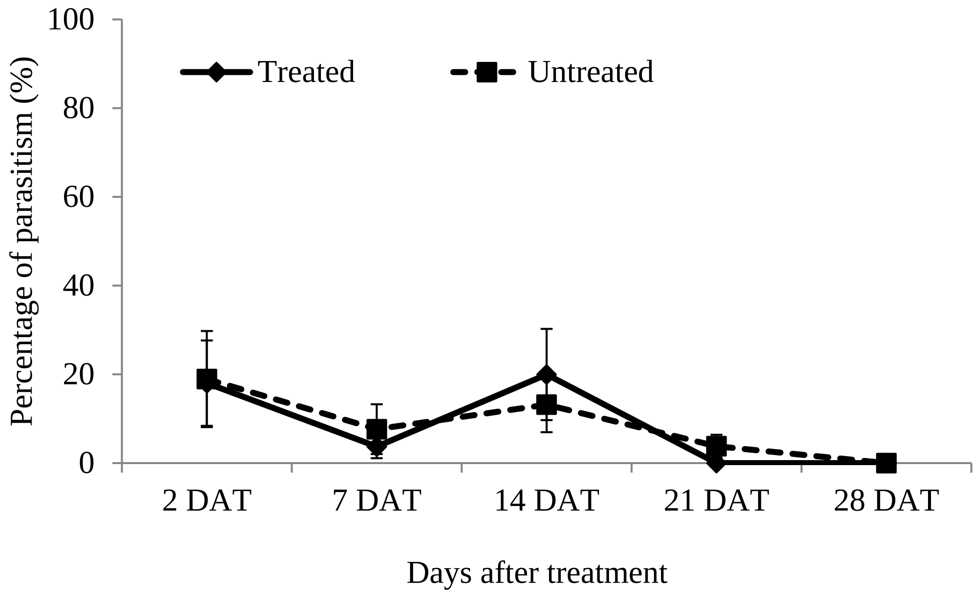



Figure 4.2. Mean percentage of stink bug eggs parasitized (%) (± SE) in 2014

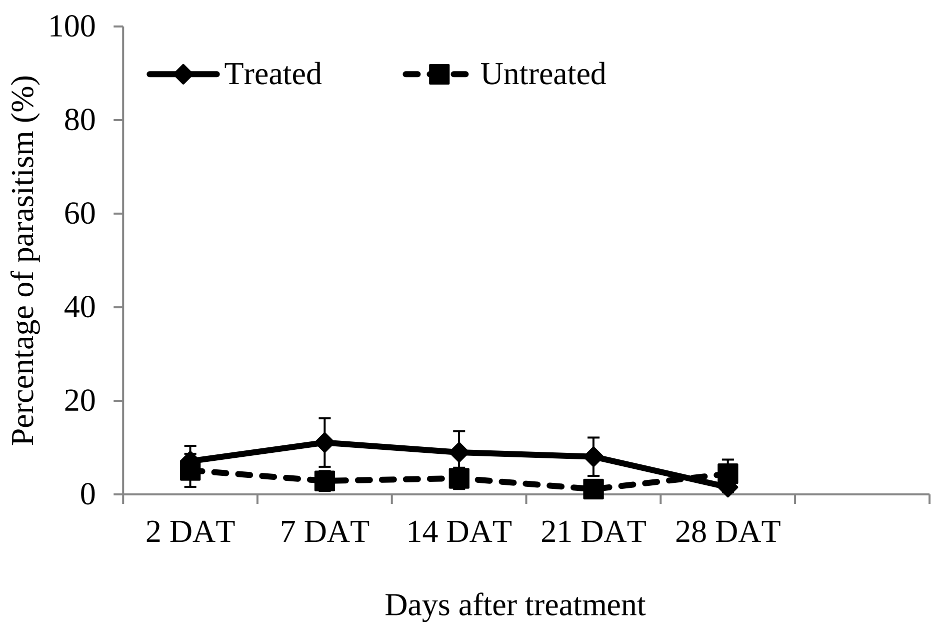



Figure 4.3. Mean percentage of stink bug eggs parasitized (%) (± SE) in 2015

### 4.4. Discussion

Results from this study showed that application of methyl jasmonate had a significant time effect on the abundance of red imported fire ants only at the 5-minute time interval in 2015. The high number of natural enemies collected at 35 DAT collaborates with previous reports (Simpson et al. 2011, Vieira et al. 2013). An increase in the abundance of plastygastrid parasitoids was reported to last for about a month on soybean plants that were treated with *cis*-jasmone (Simpson et al. 2011, Vieira et al. 2013). Possibly, the second application of methyl jasmonate led to an increase in the abundance of fire ants. Rodriguez –Saona et al. (2001) reported that the induction of herbivore induced volatiles reduces over time when only one methyl jasmonate application is made.

Methyl jasmonate treated and untreated plots had similar number of fire ants in both years except at the 5-minute point in 2015. A plausible explanation for this observation is the ability of methyl jasmonate to send signals to neighboring plants by production of predator attracting volatiles (Bruin and Dicke 2001). Further, airborne signals were proposed as being found in plant-plant interactions (Arimura et al. 2002). It is possible that soybean plants in the current study were involved in similar interactions, although this requires further investigation. Methyl jasmonate did not have an effect on parasitism rates during both soybean growing seasons. This could be due to the inability of adult parasitoids to locate food resources (Simpson et al. 2011). Moreover, treatment with methyl jasmonate does not always lead to an increase in intensity of parasitism (Simpson et al. 2011, von Merey et al. 2012). It was also proposed that platygastrid parasitoids may have challenges in locating egg cues (Vieira et al. 2013) because they use indirect cues or host searching (Moraes et al. 2005, Moraes et al. 2008). Moraes et al. (2008) further suggested that "switching-off" mechanisms for *T. podisi* attraction occurred when soybean plants had a combination of stink bug eggs and insect damage. In addition, *T. podisi* was the only parasitoid recovered in the present study and it was previously reported to respond selectively to defensive compounds of different stink bug species (Hatanaka 1993).

Regardless, the positive response of the fire ants to exogenous methyl jasmonate observed in 2015 at the 5-minute time interval can be an added advantage in the integrated pest management of stink bugs on soybeans. Cortesero et al. (2000) suggested that manipulation of plant signals could enhance biological control. This current study demonstrated that application of methyl jasmonate can enhance red imported fire ants up to one month after application. Future evaluation of intraguild predation in the presence of red imported fire ants would provide additional information on different soybean plant-insect interactions.

# CHAPTER 5: SUMMARY AND CONCLUSIONS

The most important insect pests of soybean in Louisiana include the soybean looper *Chrysodeixis includens* (Walker), velvetbean caterpillar, *Anticarsia gemmatalis* (Hübner), green cloverworm *Hypena scabra* (F.), threecornered alfalfa hopper, *Spissistilus festinus* and the stink bugs (Hemiptera: Pentatomidae). The stink bugs cause major economic losses to growers in Louisiana by seed damage and the need for repeated insecticide applications. Currently, the redbanded stink bug, *Piezodorus guildinii* (Westwood) is the most abundant stink bug in Louisiana and is capable of causing more damage compared to other species. Control of this invasive involves scouting which is followed by chemical treatment but *P*. *guildinii* is more tolerant to insecticide application in the United States.

Work in Brazil on *P*. *guildinii* demonstrated that different approaches to managing this pest can be implemented. Moreover, the management strategies can be integrated to reduce insecticide application. For these reasons, three main objectives were proposed to evaluate different management tactics on soybeans in Louisiana. The main objectives were to determine the current status of stink bug egg parasitoids and their percentage of parasitism in Louisiana. The second objective was to evaluate interactions between host plant resistance, insecticides, and biological control, and their effects on stink bugs. Lastly, the third objective was to determine effects of exogenous application of methyl jasmonate on soybean plants on natural enemies of stink bugs.

For the first objective, a total of 4,621stink bug eggs were collected. The egg parasitoid *Telenomus podisi* Ashmead (Hymenoptera: Plastygastridae) was the most abundant during the study. The parasitism of eggs was also influenced by plant structures with most of the parasitoids attacking stink bug eggs on leaves. Results from the second objective demonstrated that the soybean cultivar Pioneer 95Y20 consistently reduced pest pressure, seed damage, and increased yield. The chemical thiamethoxam performed better than the other insecticides. Results from the last study demonstrated that there was a treatment effect of methyl jasmonate on red imported fire ants collected after a 5-minute time interval in the field. However, the treatment with methyl jasmonate did not affect the percentage of parasitism rates on stink bug eggs. This was partly due to low incidences of stink bug egg clusters.

These studies demonstrated that *P. guildinii* was successfully attacked by egg parasitoids. However, emergence of these parasitoids may have been disrupted by chemical application in the second study. Host plant resistance played a significant role in reduction of stink bug pest pressure and damage. Therefore, a recommendation may be that moderately resistant soybean be incorporated in an integrated pest management (IPM) approach. In conclusion, host plant resistance should be the main pest control strategy and insecticides should be applied when economic thresholds are reached to conserve natural enemy populations.

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# VITA

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